ACTA BOTANICA NEERLANDICA

PUBLISHED BY THE

KONINKLIJKE NEDERLANDSE BOTANISCHE VERENIGING

(Royal Botanical Society of the Netherlands)

Volume 7

1958

NORTH-HOLLAND PUBLISHING COMPANY
AMSTERDAM

Digitized by the Internet Archive
in 2024

https://archive.org/details/acta-botanica-neerlandica_1957-12-24_7_1-768

Acta Botanica Neerlandica **7** (1958) 1–32

INFLUENCE OF INHIBITORS ON THE UPTAKE AND THE TRANSPORT OF CHLORIDE IONS IN LEAVES OF VALLISNERIA SPIRALIS

W. H. ARISZ
(*Botanical Laboratory, Groningen*)

(*received December 24th, 1957*)

INTRODUCTION

From previous researches it has appeared that the uptake of chloride into *Vallisneria* leaves is an active process that accumulates chloride ions in the leaf, owing to energy available in the cell or supplied when exposed (1947, 1952, 1956). The question of the absorption and the transport of the accompanying cations is not raised here. Respecting the influence of light on chloride absorption we refer to a recent publication by R. N. VAN LOOKEREN CAMPAGNE.

ARISZ showed that the uptake is a complex process consisting of various biochemical component processes. He distinguishes a process of ion uptake into the symplasm beside a secretion of ions from the symplasm into the vacuole and a transport from cell to cell in the symplasm outside the vacuoles. Both uptake and secretion are active processes, i.e. processes connected with metabolism. In how far the transport process itself may be called active will be discussed later.

In this publication the term "accumulation" will be used for a process that accumulates salt ions in the cell. For a considerable part this is accumulation in the vacuole owing to secretion of ions from the symplasm. This appears from the increase in osmotic value of the cellsap, demonstrated by A. van Schreven and A. van der Molen (ARISZ 1943, 1956). This does not exclude that in *Vallisneria* in mitochondria or in other parts of the plasm accumulation of ions can occur (cf. Robertson). It is desirable to state here that by ion transport is meant the transfer of ions both over very short distances in the cytoplasm and over longer distances in the symplasm. Transport from the medium into the cell plasm is called uptake into the symplasm. The transport mechanism has still been little investigated. The chloride ions, free or bound to carriers are easily transportable in the plasm and are not released to the medium during the transfer. This might point to the presence of an outer layer of the plasm difficult to permeate for these ions.

Of late years the view has been stressed that in many cells and tissues there are spaces in the plasm in open communication with capillary spaces in the cell wall and with the external solution so that a reversible penetration of salts from the medium into the plasm can occur. Wall- and plasmatic spaces would then form a "free space", which is calculated as "apparent free space", (Hope and Stevens, Hope and Robertson, Robertson, Butler, Lundegårdh, Burström, Hylmö, Epstein for roots and Cowie and Roberts for *Escherichia coli*). Cowie and Roberts (1955) assume "that free metabolites in this space can diffuse out, metabolites involved in the metabolic reactions of the cell are not free, but attached to some larger molecules and thereby prevented from diffusing out". Epstein (1956) suggests that also in *Vallisneria* leaves ions may be bound to larger molecules according to the concept discussed by Cowie and Roberts (1955). The problem of a free plasmatic space in *Vallisneria* will be more fully treated elsewhere. Here it may be observed that if the hypothesis is correct that the ions in the cytoplasm are bound to large molecules (Overstreet), nevertheless the complexes formed in *Vallisneria* leaves must be easily transportable in the symplasm without being released to the medium. Essentially the two representations don't differ much. In the one case uptake into the symplasm is binding to carriers present in the symplasm. In the other case it is a membrane passage at the cost of energy provided by the cell, the mechanism of which has not been explained yet, but may likewise be connected with binding to a penetrating carrier. An important difference is, however, that the free ions have a charge, whereas the complexes of ion and carrier have none (cf. p. 25).

On treating this problem other investigators have not paid attention to the transportability of the ions in the symplasm either alone or as a complex together with the carriers to which they are bound. In the discussion we shall revert to the uptake into the symplasm. Here it may be stressed that in a system consisting of various processes an uptake into the symplasm can only be physiologically demonstrated, if this process is limiting for the accumulation of ions into the cell. This sometimes seems to be the case. Arisz and Sol (1956) for instance found a considerable increase in the uptake of chloride ions into the symplasm on exposure to light of the absorbing part. This indicates that in the dark the uptake process was limiting. The uptake into the symplasm will be dependent on various factors, of which the regulation of the permeability of the membrane for cat- and anions is one and the provision of energy for the transport of ions, for the formation of carriers or for the binding to a carrier a second. Also if one uses an inhibitor which influences one of the processes involved in the uptake into the symplasm, it is to be expected that under the influence of that inhibitor the uptake into the plasm may become limiting for the accumulation of ions.

In a previous investigation (1953) the effect of inhibitors, among which 2,4-dinitrophenol and KCN on the uptake and transport processes was discussed. Only a preliminary communication has appeared hitherto. The plant material used in 1952 had developed vigorously and was very resistant, which made it capable of enduring fairly high concentrations of dinitrophenol without the protoplasm being injured. This was proved by the fresh look of the leaves at the end of the experiment, by the absence of exosmosis and by the undisturbed continuance of the transport. In this material dinitrophenol appeared to inhibit the vacuole secretion locally, but not the uptake into the symplasm and the transport. We have not yet succeeded in repeating these experiments with dinitrophenol with an entirely satisfactory result. In 1953 and subsequent years a number of other inhibitors were examined among which some giving a similar result as dinitrophenol, whereas others behaved like KCN. Here we mention only hydroxychinoline, sodium azide, KCN, sodium arsenate and uranyl nitrate.

Especially because meanwhile more experience has been obtained about the culture of *Vallisneria* and about the circumstances favourable for uptake and transport these inhibitors have given satisfactory results, which have corroborated and supplemented the results previously obtained. Especially in the spring of 1957 the *Vallisneria* material used was sufficiently resistant to endure inhibitors and consequently at the same time suitable for an operative interference to investigate the paths for transport.

In a discussion at the Conference of the Society for Experimental Biology at Groningen held on 31 March 1955, Dr. Sutcliffe made the suggestion that inhibitors may affect the transport in *Vallisneria* leaves. This might be based on an influence of the inhibitors on the transport of chlorides by the bundles. It is true these bundles are reduced in *Vallisneria* in connection with life under water, but distinct sieve tubes are present. This suggestion has given cause to investigate the parenchymatous transport and the one by bundles separatelv on their sensitiveness to inhibitors in connection with our experiments on "The path of salt transport" (ARISZ and SCHREUDER 1956). In doing so for azide a difference was found that may be interpreted in the sense meant by Dr. Sutcliffe.

METHOD

The method of investigation links up with that of previous publications, especially that by ARISZ and SCHREUDER (1956 a and b).

The first series of experiments was carried out in 1953 on plants grown in tap water in the daylight. In later years material was used, grown in artificial light in water that had been made free of salt by ion exchange resins. The plants were kept in a cellar in concrete tanks (110 by 110 cms surface and 50 cms depth), in soil mostly rich in organic manure. For the transport experiments leaf lengths were always used of 7.5 cms length and 4 mms width; 8 of such leaf lengths form a series of which after the uptake period of 24 hours, 3 series of 8 segments of 2.5 cms length were cut which were analysed for chloride. The difference in chloride per series, present at the beginning and at the end of the experiment gave the increase during the time of experimenting. The leaf lengths were destructed in nitric acid with silver nitrate. Chloride was determined according to Volhard, van Slyke and Sendroy. With the experiments the numbers are given, because experiments with material of the same age and pre-treatment give analogous results. Seeing an average of two experiments was made per week, from the numbering of the experiments an impression may be obtained on the lapse of time between the different experiments.

During transport the leaves were in closed perspex boxes, divided into compartments. The first 2.5 cms, the absorbing zone, was put into contact with the salt to be taken up, which was administered either in agar (0,01 M KCl + $CaSO_4$) or in a solution (0,001 M KCl+ $CaSO_4$). The adjoining 5 cms, the free length was either entirely kept in distilled water or between agar strips or there was a separate compartment for the middle zone. This lay in a solution or between agar strips, to which, if needed, an inhibitor could be added. It was usually prevented that in two adjoining compartments a liquid was present in both. The Difco agar used was free of chloride. The experiments with operated leaves were made in the same way as mentioned by Arisz and Schreuder. In the greater part of the experiments Mrs. van der Helm–Schreuder lent her assistance. I am much indebted to her for the accurate determinations and the extraordinary skill in the treatment of the sensitive material, for which also in case of the operated leaves reliable and reproducible data were obtained.

RESULTS

Before the influence of inhibitors on the transport is treated, the course of the transport as to time ought to be discussed. Here an experiment is being discussed on transport after 3, 6, 12 and 24 hours (Fig. 1). In the first three hours 90 μg Cl is accumulated in the

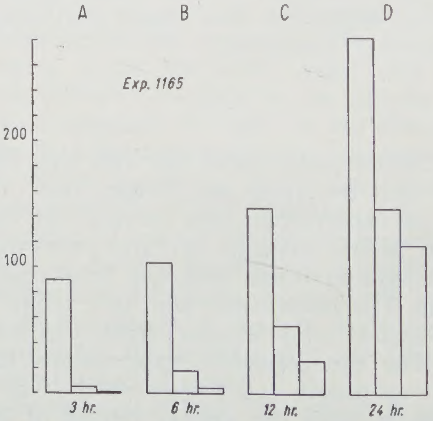


Fig. 1. Transport in the light of chloride in leaves of 7.5 cm length at 25° C. The increase of chloride after 3, 6, 12 and 24 hours for three segments of the leaves is estimated. The first zone is the absorbing zone. On the ordinate the amount present in the segments in μ g Cl.

absorbing zone, while but few chloride ions have been accumulated in the two transport zones. In the absorbing zone accumulation starts at once, the rate decreasing a little in the following hours; in the middle zone accumulation is first weak, increases constantly and does not reach its maximal rate before twelve hours have past. In the third zone hardly any accumulation can be demonstrated in the first three hours; during the next three hours it is greater and from 12 to 24 hours the accumulation in the second and third zones occurs at a fairly equal rate. In the absorbing zone the velocity is but little greater in that period. This course indicates that only after some hours the supply of chloride ions in the plasm is sufficiently great to permit of a maximal accumulation. It seems as if an induction period must elapse before the supply to each zone has reached a sufficiently high level. The length of this induction period depends on the distance over which the transport has to take place. A competition for chloride ions available for accumulation among the various zones is not to be observed in this series of experiments. This is connected with the strong uptake of ions into the symplasm in the light. Arisz and Sol (1956) did find competition when the absorbing zone was in the dark.

The experiments made here on the influence of inhibitors on uptake and transport all lasted 24 hours. On the influence of inhibitors in shorter experiments and the reversibility of the inhibition experiments are desirable. Data on the influence of external factors on the uptake process for short periods are being gathered. Such data on the transport are much more difficult to obtain as the rate of transport has to be determined from data on the quantity of ions fixed by accumulation in various zones.

Influence of Sodium azide on the uptake and the transport of chloride ions

Experiments 852, 854 and 857 were made in 1953 on leaves, the absorbing zone of which (2.5 cms) was in the dark and the free leaf part (5 cms) exposed to light. From these experiments it appears that 1.5×10^{-4} M. azide added to the agar with KCl causes a considerable decrease in accumulation of chloride in the absorbing zone (Fig. 2). As is known from a publication by Arisz and Sol, uptake and transport in leaves exposed in this way will be comparatively weak, because in the dark the uptake into the symplasm becomes limiting for the accumulation in the various zones. Also when azide is added to agar in contact with the middle zone, an inhibition of the accumulation is obtained, but this continues more or less limited to the place of administration. As the middle zone does not take up the chloride ions direct from the medium, but via the symplasm from the absorbing part, this means that azide is capable of inhibiting the accumulation in the vacuole locally. The transport through the symplasm to the adjoining length in these experiments is but slightly inhibited by azide, sometimes not at all (exp. 854 B, 857 B). This means that in the zone treated with azide the transfer of ions in the symplasm continues in a normal way, while the active secretion process causing accumulation in the vacuole is prevented. On administration of azide to the absorbing part the second and third zones forming together the free part can continue accumulating chloride (experiment 854) the uptake from the medium and the transport not being inhibited by azide. Sometimes (experiments 852 and 854) the secretion of

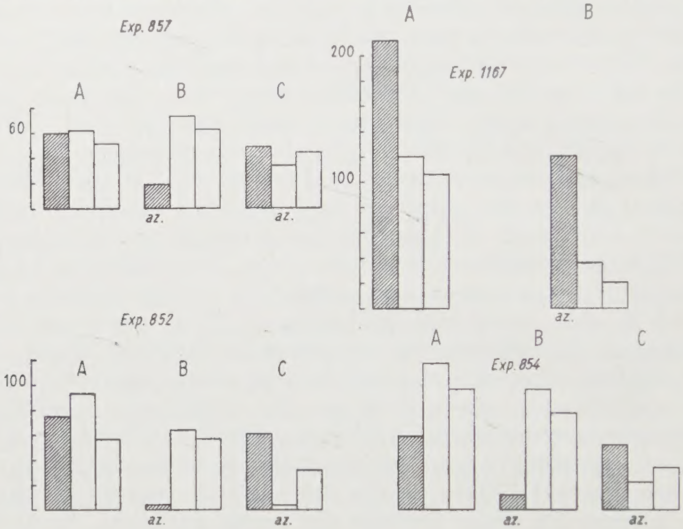
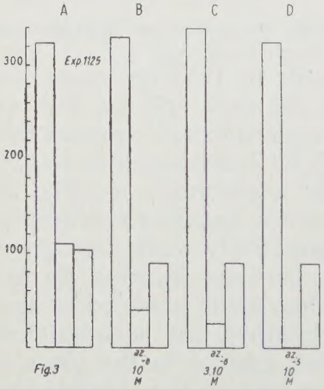


Fig. 2. Influence of addition of sodium azide to the first or the second zone of the leaflengths. The absorbing zone is in the dark. Exp. 857, 852, 854 uptake of 0.005 M KCl + $CaSO_4$ 1.5 × 10^{-4} M azide. Exp. 1167 0.001 M KCl + $CaSO_4$, 10^{-5} M azide. 24 hours 25° C.



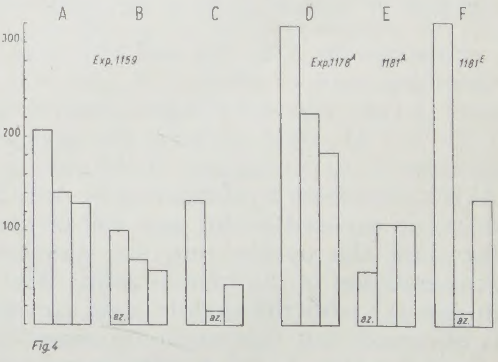


Fig. 3. Pretreatment 48 hours in the dark. Influence of addition of sodium azide in B, C and D to the middle zone of the leaf. Uptake of 1/100 M KCl with $CaSO_4$ by the absorbing zone. Conc. of azide in B 10^{-6} M in C 3.10^{-6} M and in D 10^{-5} M. Exp. 1125, 24 hours 25° C.

Fig. 4. Influence of addition of sodium azide in B and E to the absorbing zone in C and F to the middle zone. Pretreatment 24 hours in the dark. Uptake of 0.001 M KCl + $CaSO_4$ in the light. 10^{-5} M azide is administered to the absorbing zone in B and E, to the middle zone in C and F. Two experiments 1159 and 1178 + 1181.

chloride has slightly decreased in these zones. This indicates that azide can also influence the transport process direct or indirect. These experiments made in 1953 have been repeated in 1956 and 1957. The uptake and the transports were much greater in these experiments, owing to the plants now being grown in water poor in salt while the uptake into the absorbing zone occurred in the light except in experiment 1167 (Fig. 2), where azide was administered to the absorbing zone which was in the dark, and inhibited the accumulation in all zones, especially in the free part.

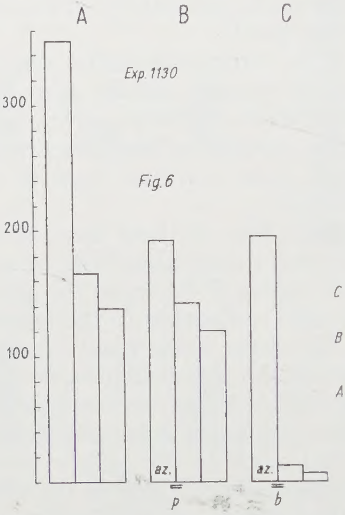
In experiment 1125 (Fig. 3) in which the entire leaf was exposed to light, administration of azide to the middle zone in increasing concentrations causes a stronger local inhibition of the accumulation, whereas the accumulation in the absorbing zone, the transport to and the accumulation in the third zone continue equally great. Here therefore the effect of azide is local.

In experiments 1159 and 1181 (Fig. 4) there have been compared the influence of azide administered to the absorbing or to the middle zone. In these experiments the action of azide on the absorbing zone is not limited to this zone, but also noticeable in the second and third zones be it in a less degree. The same holds good on administration to the second zone, when likewise the accumulation in the third zone is inhibited. The inhibition in 1181 F being very strong in the middle zone, not noticeable at all in the absorbing zone and weak in the third zone it is obvious to assume that azide in the middle zone sometimes influences the transport to the third zone. Azide in the absorbing part may inhibit the transport to the second and third zones.

Transport by tissue bridges

As communicated in the introduction, some experiments have been made to trace whether azide affects the transport in the vascular bundles and in the parenchymacells. In a previous publication (Arisz and Schreuder) it was investigated along what paths transport of chlorides can occur in the *Vallisneria* leaf. Just as in previous experiments (Arisz and Oudman) on the transport of asparagine in these experiments the transport in the parenchyma or in the bundles was prevented by locally removing the parenchyma or the bundles over a distance of 4–16 mms (Fig. 5). As a rule for transport by parenchyma bridges (B), some parenchyma paths were left intact and all intervening bundles removed over a certain distance. In the bundle bridges (C) only the central vascular bundle was left intact, while over a distance of 4, 8 or 10 or 16 mms the tissue on both sides of this median bundle was removed over the entire width. Seeing that in doing so the median bundle had to remain intact as much as possible, some parenchyma cells in close contact with it, have not been removed, so that in case of a bundle bridge it cannot be said there was an exclusive transport through sieve tubes, though they will doubtless have provided the most important path. From the experiments by Arisz and Schreuder it is known that after a pretreatment of 24 hours after making the injury, the chloride transport has been sufficiently recovered as well by a parenchyma bridge as by a bundle bridge. The one vascular bundle left is capable of an intensive transport. The zone of 4–16 mm length over which the operation extended was not included in the analysis of the intact leaves (A) either. Also in longer isolated bundles (8, 10 and 16 mms length) a but slightly weakened transport is to be shown (Fig. 10).

In experiment 1130 (Table 1 and Fig. 6) a normal transport (A)



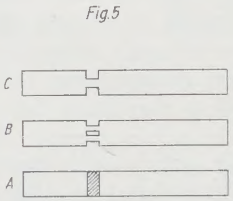


Fig. 5. A is an intact leaf. The shaded zone is not analysed. B two bridges of parenchyma cells. C one bridge of a bundle.

Fig. 6. Influence of azide administered to the absorbing zone in B and C on the transport. In B a bridge of parenchyma p, in C a bundle bridge b. Exp. 1130, 24 hours, 25° C.

TABLE I

Influence of sodium azide on the uptake and transport of Cl in intact leaves, in leaves with a parenchyma bridge and in leaves with a bundle bridge. Length of the bridges 4 mm. I is the absorbing zone, II middle zone, III end zone of a leaf length. Each zone is 2,5 cm long and 0,4 cm wide. Amount of Cl in μg Cl/8 leaflengths 2.5 . 0,4 cm; 25° C. 24 hours. Exp. 1130.

	Cl content in μg Cl present in zone			Cl increase in μg during uptake		
	I	II	III	I	II	III
	263	270	—	—	—	—
After pretreatment in water	absorbing	middle	end			
after uptake of:	zone	zone	zone	—	—	—
0,001 M KCl + $CaSO_4$ . . .	618	433	405	351	166	138
idem + 10^{-5} azide. . . .	454	398	383	187	131	116
idem + 10^{-5} azide. . . .	462	419	390	195	152	123
(parenchyma bridge)						
idem + 10^{-5} azide. . . .	447	277	263	180	10	— 4
idem + 10^{-5} azide. . . .	476	284	284	209	17	17
(bundle bridge)						

has been compared with the effect of azide on a transport through parenchyma bridges, in which the bundles have been removed over a 4 mm distance (B) and on a transport by one median isolated bundle, the tissue on either side of this bundle being removed over a 4 mm distance, both bundles and parenchyma (C). The two series have been carried out in duplicate. It is a striking fact that in C the transport via the bundle bridge is greatly decreased by azide, whereas that via the parenchyma in B is hardly reduced. This follows from the slight accumulation of chloride in the cells of the free length in C. The parenchymatous transport is apparently not being inhibited by azide, but the transport via the sievetubes is. This phenomenon will be referred to as the "Sutcliffe effect" (cf. p. 3).

In experiment 1134 with parenchyma bridges and 1135 with bundle bridges this phenomenon has been further investigated. In experiment 1134 Fig. 7 A is an intact leaf, B a leaf where at 25 mms distance from the one end the bundles have been removed over a distance of 4 mms so that transport in the bridges only occurs through parenchyma cells. C is an intact leaf treated with 10^{-5} M sodium azide to the absorbing part. The absorbing part in D has likewise got azide, the bundles being removed over a distance of 4 mms. The operation zone of 25 to 29 mms has not been included in the analysis neither in A, nor in B, C and D.

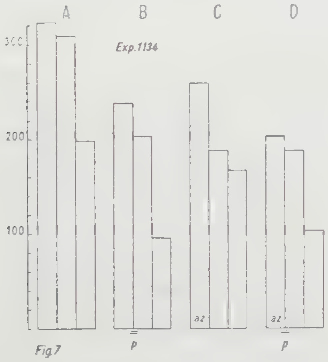


Fig. 7. A transport in an intact leaflength. Influence of a parenchyma bridge on the Cl transport. (B and D). In C and D 10^{-5} M azide is administered to the absorbing zone. Length of the bridges 4 mm. 24 hours, 25° C. uptake of 0.001 M KCl + $CaSO_4$ in the light. Exp. 1134.

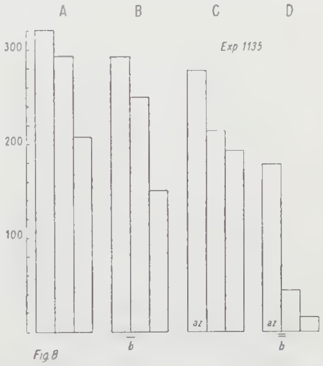


Fig. 8. The difference with fig. 5a is that the bridges of B and D are bundle bridges. Exp. 1135, 24 hours, 25° C.

After 24 hours an increase in chloride is found, indicated in Fig. 7. In agreement with previous results (Arisz and Schreuder) it appears that parenchyma bridges of 4 mm length permit of a proper transport of chloride ions. Owing to the operation the accumulation in B in all three zones is weaker than in the intact leaf. In C azide gives a slight local inhibition in the absorbing zone of the intact leaf, the transport to the free leaf part being only slightly affected. If the transport to the free part occurs via parenchyma bridges (B and D) administration of azide to the absorbing part has only a slight influence.

The result of experiment 1135 (Fig. 8), where the transport via the zone of 25 to 29 mms occurs through one isolated bundle only, is different. The transport through the isolated bundle in series B is almost equally strong as the one in the intact leaf. If one considers that here mainly the sieve tubes are active, these elements must permit of a comparatively stronger transport than the parenchyma cells.

Azide added to the absorbing part gives a slight inhibition in all zones in series C in comparison with series A. It is a striking fact that in series D where beside the inhibition by azide in the absorbing zone transport must moreover take place through one bundle bridge, just like in Fig. 6 C, the transport to the free part is greatly inhibited, as appears from the slight accumulation. From these experiments it appears that transport via the bundle bridge is inhibited by azide but that via the`parenchyma bridges is not.

Influence of the length of the bridge

Fig. 9 gives a number of results about the influence of the length of the parenchyma bridge on the transport. It appears that bridges of 8 and 16 mms (B and C) transport the chlorides considerably worse than a bridge of 4 mm length (A). Also the absorbing zone has a weaker accumulation than normal as a result of the operation. If the middle zone is treated with azide, the accumulation in that zone is weak, the transport to the third zone also being inhibited, so that there the accumulation only attains a low level (D, E and F). Therefore using bridges longer than 4 mms is undesirable in case of parenchyma bridges.

Contrasted with this is the effect of a longer bundle bridge (Fig. 10). Still with an 8 mm bridge there is a fairly normal transport to the free part. The accumulation in the absorbing part continues quite normal.

From experiment 1146 (Fig. 11) it may be seen that the middle zone to which azide has been administered, has a slight accumulation while both at 4, 8 and 16 mm bundle bridges the transport to the third zone and the accumulation there is equally great or even a little greater than in the intact leaf. Here therefore in spite of addition of azide to the middle zone, transport from the second to the third zone occurs with an unaltered strength. It cannot be indicated here whether this transport occurs in the symplasm or in the bundle.

Experiment 1151 (Fig. 12) gives the influence of azide in leaflengths with a bundle bridge of 10 mm length. The greater length of the bridge enables us to locally administer azide, besides to the absorbing part (B) and to the middle zone (D), also to the 10 mm bridge (C).

In series C azide, locally administered to the isolated bundle causes only a slight decrease in accumulation in the adjoining middle zone, the third zone having an almost normal chloride accumulation.

If, however, in series B azide is administered to the absorbing zone, the transport to the second and third zones is considerably inhibited ("Sutcliffe effect"). Azide added to the middle zone (D) inhibits the accumulation in this zone, the accumulation in the third zone being lower than without azide (A) in this case.

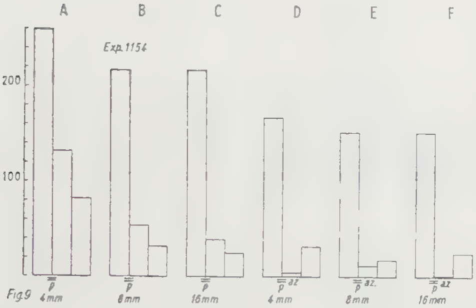


Fig.9

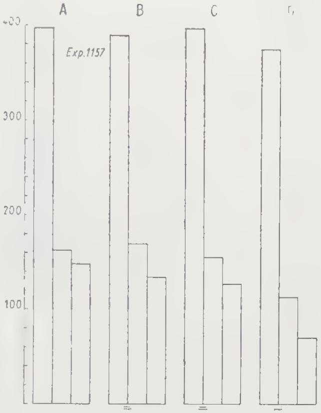


Fig 10

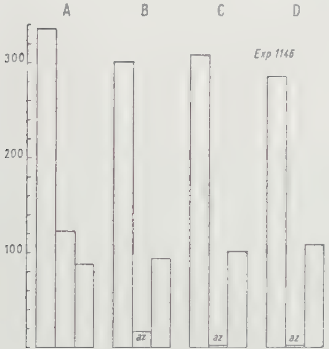


Fig 11

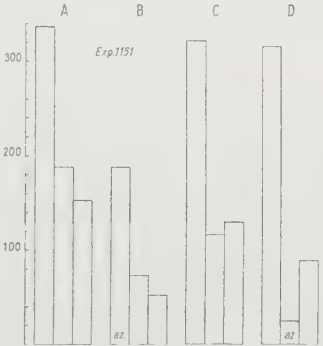


Fig 12

Fig. 9. Influence of the length of the parenchyma bridge on the transport of chloride. In A and D 4 mm, in B and E 8 mm, in C and F 16 mm length. In D, E and F 10^{-5} M azide is administered to the middle zone. Uptake of 0.01 M KCl + $CaSO_4$ in the light; 24 hours, 25° C. Exp. 1154.

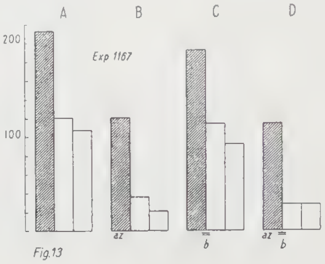
Fig. 10. Influence of the length of the bundle bridge on the transport of chloride. In B 4 mm, in C 8 mm and in D 16 mm. Uptake of 0.01 M KCl + $CaSO_4$ in the light 24 hours, 25° C. Exp. 1157.

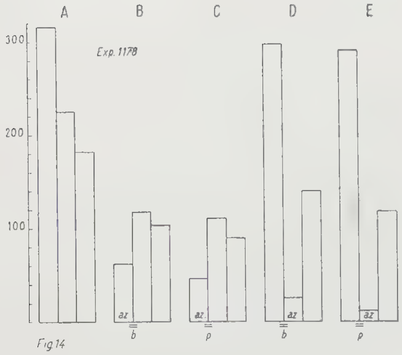
Fig. 11. The difference with fig. 10 is the administration of azide 10^{-5} M to the middle zone in B, C and D. Exp. 1146.

Fig. 12. Influence of bundle bridges of 10 mm length and azide on the transport of chloride. In B azide is administered to the absorbing zone, in C to the isolated bundle bridge and in D to the middle zone. Uptake of 0.01 M KCl + $CaSO_4$ in the light, 24 hours, 25° C. Exp. 1151.

An experiment in which the absorbing zone was in the dark, gave the same results. In experiment 1167 (Fig. 13) the absorbing zone is in the dark and the rest of the leaf in the light. Azide administered to the absorbing zone (B) again gives inhibition of the accumulation in all leaf zones. In the presence of a bundle bridge the effect is fairly equal.

Experiments 1178 and 1181 (Fig. 14, 15) are supplementary. They have been made in successive weeks on particularly good material from the same basin, so that the absorbed quantities of chloride are comparable and the experiments supplementary. In experiment 1178 the azide inhibition in bundle- and parenchyma bridges of 4 mm length has been compared. In B and C azide has been given to the absorbing length, in D and E to the middle zone. In this experiment the nature of the bridge has no influence. Azide in the absorbing zone also inhibits the accumulation in the other zones. When azide is





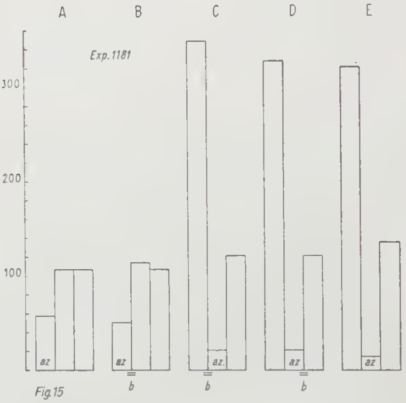


Fig. 13. Influence of 10^{-5} M azide and bundle bridges of 10 mm length. The absorbing zone is in the dark. The free part of the leaf is exposed to light. Uptake of 0.001 M KCl + $CaSO_4$, 24 hours 25° C. Exp. 1167.

Fig. 14. Influence of azide and bridges on the chloride transport. In B and D bundle bridges of 4 mm length, in C and E parenchyma bridges of 4 mm length. Azide is administered to the absorbing zone in B and C, to the middle zone in D and E. In this experiment young *Vallisneria* leaves were used. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 24 hours, 25° C. Exp. 1178.

Fig. 15. Influence of azide on the transport through bundles. In A and B azide is administered to the absorbing zone, in C, D and E to the middle zone. A 4 mm bundle bridge is situated in B and C between the absorbing and the middle zone, in D between the middle and the third zone. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 24 hours, 25° C. Exp. 1181.

administered to the middle zone the inhibition of the accumulation in the adjoining zones is slight (also confer experiment 1181 C and E).

In experiment 1181 azide administered to the absorbing zone (A and B) causes inhibition of the accumulation in all zones. A is a transport inhibited by azide without operation, in B a bundle bridge is present. The latter does not influence transport and accumulation in the free length. In C, D and E azide has been added to the middle zone, in E no operation has been performed, in C there is a bundle bridge at a distance of 25 to 29 mms from the end. In D the bundle bridge lies at the end of the middle zone, 50 to 54 mms from one end. The accumulation in the third zone does not show any influence of the situation of these bridges. In series A and B and in experiment 1178 series A, B and C the absorbing zone was kept in a solution and the free zones (second and third zones) between agar strips. This has prevented the chloride administered to the absorbing zone from getting into the medium of the second and third zones by an experimental error. In the method applied, in which the absorbing zone is brought into a solution (0,001 M) (KCl + CaSO$_4$) and the tiny apertures in the partition between the compartments have been closed with vaseline, the free length lying between agar strips, there is no other path of transport for the chloride ions than through the leaf. In experiment 1181 C, D and E and 1178 D and E azide was given to the middle zone in a solution. The unaltered uptake into the first zone proves that azide has only acted locally.

Influence of KCN on the uptake and the transport of chloride ions

In 1953 Arisz demonstrated that KCN greatly inhibits the uptake of chloride into a leaf zone. In transport experiments, in which KCN was administered to the absorbing zone, it appeared that both in the absorbing zone and in the free part of the leaf accumulation of chloride was inhibited (Fig. 16). If, however, KCN was only given

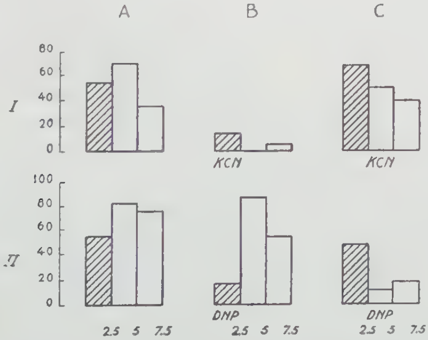


Fig. 16. Influence of KCN 3.10^{-4} M and 2.4-Dinitrophenol 10^{-4} M on the uptake and the transport of chloride. In all experiments absorption of 0.01 M KCl + CaSO$_4$. The absorbing zone is in the dark and the free part in the light. (From Arisz 1956).

to the middle zone, neither the uptake nor the accumulation of chloride was inhibited in this zone. From these data important conclusions could be drawn. The lack of influence of KCN administered to the middle zone shows that the accumulation of chloride ions is not inhibited by KCN, the transport experiencing no influence either.

The strong inhibition of uptake and transport, when KCN is administered to the absorbing zone, can therefore not be based on a local inhibition of the accumulation and inhibition of the transport from cell to cell. The accumulation of chloride ions being inhibited both in the absorbing zone and the free zone, this must be due to an inhibition of the uptake of ions into the symplasm.

If a transport of chloride takes place outside the plasm via the cell walls of the absorbing zone to those of the free zones, it would be expected that also on administration of KCN to the absorbing zone this wall transport would continue. KCN being only administered to the absorbing zone, the free leaf part would take up this chloride supplied by the cell walls into the symplasm and accumulate it in their vacuoles. It appears, however, that this does not occur. This speaks against chloride transport via cell walls outside the plasm.

This also appears from the continuation of the transport to and the accumulation in the middle zone on local administration of KCN to this zone. For, KCN administration to the middle zone would certainly have inhibited the uptake of chloride supplied via the cell walls into the plasm of this zone, just as it does in the absorbing zone. Also the accumulation in the free part not inhibited by KCN indicates that no transport of chloride ions takes place from the medium solution through the cell walls to the free zones. This is the physiological proof that transport from cell to cell must take place through a symplasm. The symplasmatic transport and the accumulation in the vacuole is not influenced by KCN in this experiment, whereas the uptake into the symplasm is.

It was desirable that the small number of experiments carried out in 1952 under particularly favourable conditions with perfect *Vallisneria* material should be extended. It was disappointing that the less resistant material to be disposed of then, did not permit of a conclusive corroboration of the result obtained, though in a few cases a similar effect was obtained with arsenate.

In 1956 and 1957 the experiments were resumed. The inhibition of uptake and transport on administration of KCN to the absorbing part occurred then in all experiments without an exception, but when KCN was administered to the middle zone, there was a pronounced decrease in accumulation in that zone. In April 1957 we succeeded in recovering the effect found in 1952 in still young plants cultivated in favourable circumstances. It is a striking fact that only plants from one concrete basin showed the phenomenon looked for, while plants from an adjoining basin which were older, did show inhibition of accumulation by KCN in the middle zone. It has repeatedly appeared in the experiments made with *Vallisneria* during these years that the previous history and the age of the plants is determining for the way in which the biochemical processes of uptake and secretion of chlorides progress. With various results obtained with different material it cannot be said that one result is correct and the other wrong, but both may be right, but only hold good for material that underwent a special pre-treatment. The material of the south east basin reacted in 1957 not only on KCN in the way discussed (Fig. 19), but completely analogously on uranyl nitrate (Fig. 26, 27), and arsenate (Fig. 24). On the contrary azide, as already discussed (Fig. 15) did give with this same material an inhibition of the accumulation on administration to the middle zone, but no "Sutcliffe effect". So azide and KCN react on the same material with a different effect. Experiments simultaneously made with material from the south west basin which was older, did give an inhibition of the accumulation in the middle zone with KCN.

Though both results with KCN are correct, the behaviour rediscovered in 1957 is of a particular theoretical importance, because in this case KCN only inhibits the uptake of ions into the system and not the accumulation in the vacuole, which proves that the transport of ions does not occur in the walls, but in the symplasm.

We have distinguished wall and plasm as separate paths of transport. Of course it is conceivable that especially in younger walls such a distinction is not permitted, as in these there is also cytoplasm in the wall. So a safe conclusion is that a wall transport not controlled by the cytoplasm, is not present in *Vallisneria.*

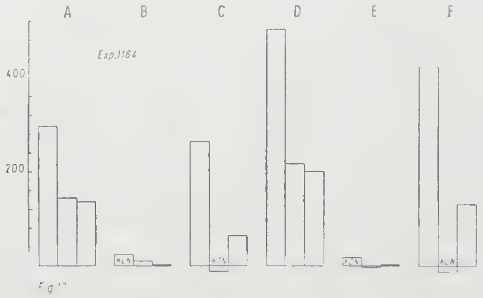
We will now discuss the experiments with KCN, arsenate and uranyl nitrate separately.

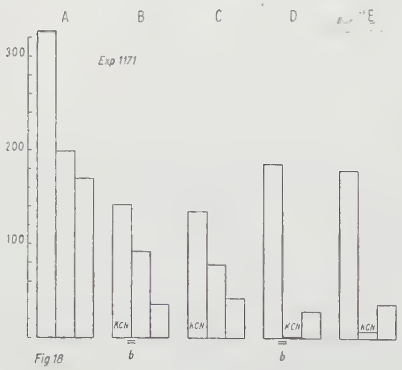
Experiments with KCN

In experiments 1164 and 1171 the effect already discussed was obtained that KCN administered to the middle zone gives a distinct local inhibition of the accumulation, whereas on administration to the absorbing zone an inhibition of the accumulation occurs in all zones. Experiment 1164 (Fig. 17) has been made with and without addition of sucrose to all zones of the leaf. KCN was used in concentration 10^{-5} M. Under the influence of sucrose the uptake into all zones increased greatly. The total uptake into the three leaf zones increases from 575 to 915 μgr. Cl. On the inhibiting action of KCN sucrose addition has no specific influence. At this KCN conc. (pH 7) the uptake is slight, the transport almost entirely inhibited. Addition of KCN to the middle zone causes a complete local inhibition of the accumulation, whereas in the adjoining zones the accumulation is slightly inhibited. To this phenomenon we will revert in the discussion.

In experiment 1171, likewise made with addition of sucrose and a KCN conc. 3.10^{-6} M a bundle bridge is present in B and D. From Fig. 18 it appears that these bridges have hardly any perceptible influence on the inhibition by KCN and the accumulation in the free part. Here too (D and E) administration of KCN to the middle zone causes a strong local inhibition and at the same time a decrease in accumulation in the two adjoining zones. Any effect due to the isolated bundle bridge is not present.

Experiment 1176 (Fig. 19) gives the result of administration of KCN 5.10^{-6} (pH 7) to leaflengths with isolated bundle or with parenchyma bridges. As a rule the accumulation is a little weaker in the case of parenchyma bridges. The bridges always have a length of 4 mms. In these experiments the perspex boxes were divided into three compartments by partitions so that KCN could only act on one certain zone. It is astonishing that in spite of the presence of large intercellular spaces in the leaf, the action of KCN is so strongly localised to the spot where it has been administered. In this experiment a series of leaves is omitted in which KCN has been administered,





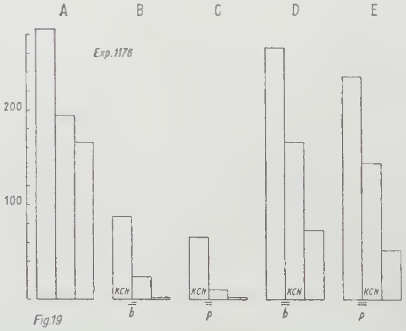


Fig. 17. Influence of KCN on the uptake and the transport of chloride. In D, E and F sucrose 0.05 M is administered to all zones of the leaves. In B and E KCN 10^{-5} M administered to the absorbing zone, in C and F to the middle zone. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 25° C. Exp. 1164.

Fig. 18. Influence of KCN on the uptake and the transport of chloride. In B and C KCN is administered to the absorbing zone, in D and E to the middle zone. In B and D bundle bridges of 10 mm length. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 25° C. Exp. 1171.

Fig. 19. Influence of KCN on the uptake and the transport of chloride. KCN 5.10^{-6} M, pH 7 is administered to the absorbing zone in B and C, to the middle zone in D and E. In B and D a 4 mm bundle bridge, in C and E a 4 mm parenchyma bridge. Uptake of 0.001 M KCl + $CaSO_4$ in the light, 25° C. Exp. 1176.

but no operation has been performed. As a result it cannot be said in this case whether the difference in accumulation of the first and third zones in D and E with respect to A is due to the operation or to a KCN influence.

It clearly appears from D and E that KCN administered to the middle zone gives hardly any inhibition of the accumulation in that zone. This is in contrast with the two preceding experiments and corresponds with the result obtained in 1952. To be sure this experiment was more successful than the one in 1952, because uptake and transport are much greater here. In this experiment with plants normally treated the total uptake of chloride was four times greater than in 1952. This is due both to the way of exposing to light and to the cultivation of the material.

Experiments with arsenate

A number of experiments have been carried out with arsenate; some with uptake in the dark and transport zones exposed to light will be discussed here first. Experiment 845 (Fig. 20) gives the same picture for arsenate as figs. 16 and 19 for KCN, i.e. arsenate does not inhibit when administered to the middle zone. Experiments 858 and 1063, however, give the deviating behaviour that arsenate also acts inhibitingly in the middle zone on the accumulation in that zone.

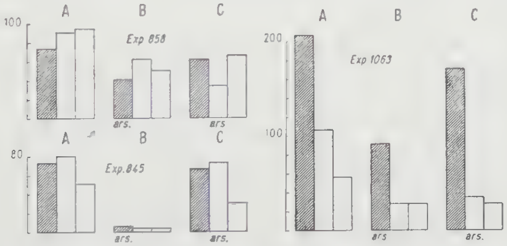


Fig. 20. Influence of sodium arsenate on the uptake and the transport of chloride. Uptake of 0.005 M KCl + $CaSO_4$ in exp. 858 and 845 and of 0.01 M KCl + $CaSO_4$ in exp. 1063, 25° C. Conc. of arsenate in all experiments 10^{-5} M. The absorbing zone is in the dark, the free part in the light.

Experiment 1163 (Fig. 21) on the inhibition with arsenate has been made simultaneously with experiment 1164 (Fig. 17), in which KCN was administered. The right of the figure (D, E and F) contains a repetition of the experiment with the only difference that sucrose was added to all zones. The results of Fig. 21 and Fig. 17 are strikingly identical. In both administration of sucrose increases uptake and transport, the inhibitions continuing to be equal.

Experiment 1156 (Fig. 22) gives the results of various concentrations of arsenate in operated leaves. In all leaf lengths there is a bundle bridge of 10 mm length. Arsenate has always been administered to the absorbing zone. It inhibits the accumulation in all zones proportional to the concentration.

In experiment 1170 (Fig. 23) the influence of a 10 mm bundle bridge has been investigated, arsenate in a 3.10^{-6} M conc. being given to the absorbing zone in series B and C or to the middle zone in series D and E. There is no specific influence of the bundle bridge, the inhibition by arsenate being in entire correspondence with those in the previous experiments. It is a striking fact that arsenate in D and E has not only got a local effect on the accumulation in the middle zone, but also clearly inhibits the accumulation in the first and third zones. Inhibition of the accumulation in the first zone does not occur in all experiments. In experiment 1177 (Fig. 24) with arsenate as an inhibitor which was carried out simultaneously with experiment 1176 (Fig. 19) with KCN as an inhibitor on material from the same basin (south east basin), a perfectly identical effect was found for arsenate as has already been discussed for KCN. Here too arsenate administered to the absorbing zone gives inhibition of uptake and transport, whereas it gives hardly any inhibition, if administered to the middle

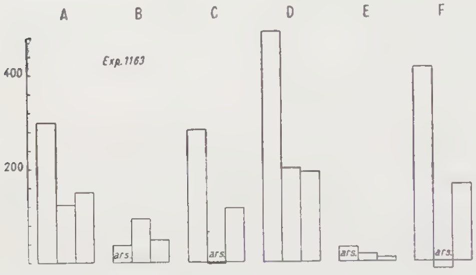


Fig21

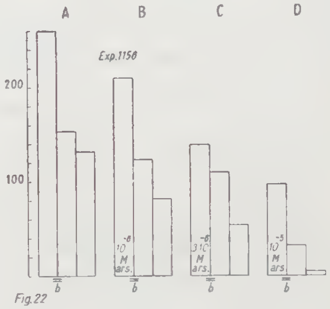


Fig. 22

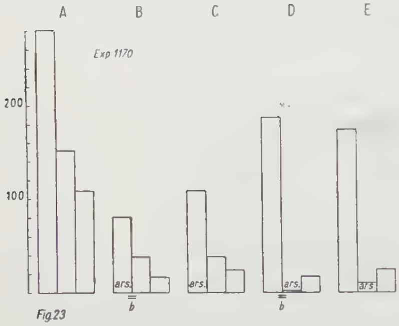


Fig23

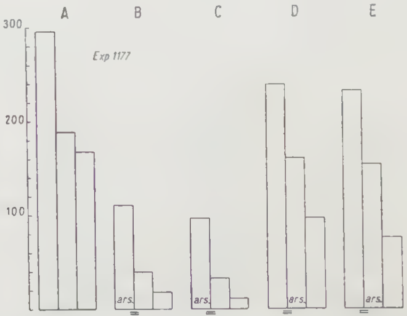


Fig24

Fig. 21. Influence of sodium arsenate 10^{-5} M, pH 7 on the uptake and the transport of chloride. In D, E and F sucrose is administered to all leaf zones. Uptake of 0.001 M KCl + $CaSO_4$, 25° C. Exp. 1163.

Fig. 22. Influence of different conc. of sodium arsenate. In all series a bundle bridge of 10 mm length. Conc. arsenate in B 10^{-6} M, in C 3.10^{-6} M, in D 10^{-5} M. Exp. 1156.

Fig. 23. Influence of sodium arsenate 3.10^{-6} M, pH 7. In B and D bundle bridges of 10 mm length. Uptake of 0.001 M KCl + $CaSO_4$, 25° C. Exp. 1170.

Fig. 24. Influence of sodium arsenate 5.10^{-6} M on uptake and transport of Chloride. In B and D a bundle bridge of 4 mm length, in C and E a parenchyma bridge of 4 mm length. Uptake of 0.001 M KCl + $CaSO_4$, 25° C. Exp. 1178.

zone. These effects are equal for bundle and parenchyma bridges. The slighter accumulation in the third zone will presumably be connected with the operation.

It should be mentioned here that in some experiments the effect of arsenate was very weak. This was likewise the case in some uptake experiments with arsenate on 2.5 cm leaf lengths. Such a lack of effect of arsenate is to be expected, when the material is rich in phosphate. Addition of phosphate to the medium also prevents the inhibition by arsenate. In agreement with this is the fact that the uptake of phosphate in *Vallisneria* is not inhibited at all by low arsenate concentrations.

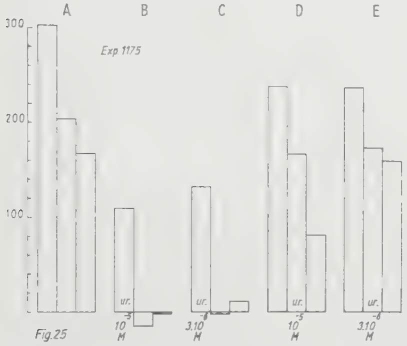


Fig.25

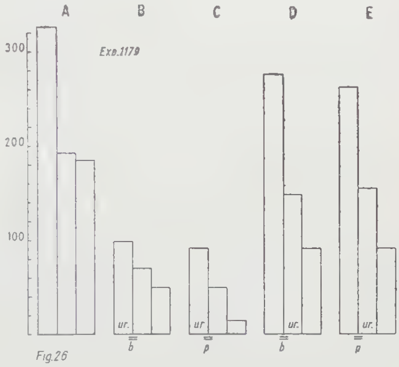


Fig.26

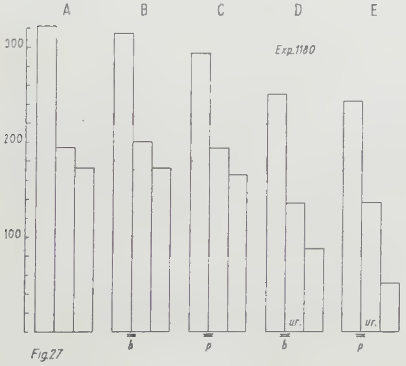


Fig.27

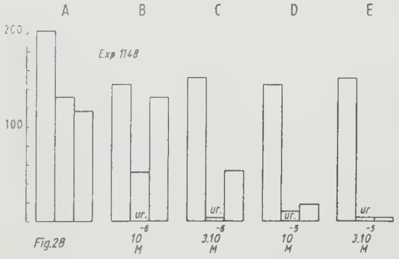


Fig.28

Fig. 25. Influence of uranyl nitrate on uptake and transport of chloride. In B and D a conc. of 10^{-5} M uranyl nitrate, in C and E 3.10^{-6} M. The inhibitor is administered in B and C to the absorbing zone, in D and E to the middle zone. Uptake of 0.001 M KCl + Ca sulfate, 25° C. Exp. 1175.

Fig. 26. Influence of uranyl nitrate 2.10^{-6} M on uptake and transport of chloride. In B and D a bundle bridge of 4 mm length, in C and E a parenchyma bridge of 4 mm length. Uptake of 0.001 M KCl + Ca sulfate 25° C. Exp. 1179.

Fig. 27. Uranyl nitrate administered to the middle zone in D and E. B and D with bundle bridges (4 mm length). C and E with parenchyma bridges (4 mm length) Exp. 1180 with same material as 1179.

Fig. 28. Influence of uranyl nitrate administered in the middle zone on different concentrations. Uptake of 0.01 M KCl + Casulfate, 25° C. Exp. 1148.

Experiments with uranyl nitrate

Some experiments have been made on the inhibition by uranyl nitrate. A few were carried out on the same material as experiment 1177 with arsenate and 1176 with KCN. They gave identical results.

Experiment 1175 is an experiment with intact leaf-lengths and two different concentrations of uranyl nitrate 10^{-5} M and 3.10^{-6} M (Fig. 25).

Uranyl nitrate administered to the absorbing leaf zone inhibits the accumulation in this zone and prevents the transport to the free leaf part. Uranyl nitrate administered to the middle zone does not influence the accumulation in that zone. In D and E the accumulation in the absorbing zone shows a very slight inhibition; that in the third zone is clearly inhibited in D (10^{-5} M), but in E (3.10^{-6} M) hardly at all.

Experiments 1179 and 1180 (Fig. 26, 27) have been carried out with the same material. In this case uranyl treatment occurred in intact and in operated leaves. In both experiments there was a bundle bridge in B and D and a parenchyma bridge in C and E, all bridges of a 4 mm length. The bridges have no effect on the results. The uptake of the intact leaves is fairly equal in the two series, so that we may also compare the other series of these two experiments. Uranyl nitrate (2.10^{-6} M) administered to the absorbing zone gives inhibition of the accumulation in all zones. Administration to the middle zone (exp. 1180 compare D and E with B and C) shows a slight decrease in accumulation in all zones. The inhibiting effect of uranyl (2.10^{-6} M) administered to the absorbing zone is about 70 %, that in the middle zone about 20 %.

In experiment 1148 (Fig. 28, B) with material of a different culture uranyl nitrate 10^{-6} M clearly inhibited the accumulation in the middle zone without inhibition occurring in the third zone. Only at a higher concentration transport to the third zone was inhibited as well.

It is a striking fact that at all concentrations of uranyl nitrate the accumulation in the absorbing part was more or less inhibited. To this fact we will revert in the discussion.

DISCUSSION

From the researches on *Vallisneria* leaves it has appeared that the uptake of salts consists of a number of processes in which the ions first enter the plasm, are transported there over a longer or shorter distance and are finally accumulated in parts of the plasm or in the vacuoles.

When we compare uptake processes in cells with and without vacuole, we should realise that on uptake into a cell without vacuole we only think of uptake into and binding to the plasm, whereas in a cell with vacuole uptake often implies accumulation in the vacuole. The two processes might progress more or less separately.

Separation of the uptake into the symplasm from the secretion into the vacuole

The principle of the analysis of the uptake processes carried out on *Vallisneria* is based on transport experiments, in which the influence of an exterior factor on the accumulation in a zone which takes the chloride ions direct from the medium, is compared with the one in an adjoining part which has to obtain the ions from the absorbing part. In both zones accumulation in the vacuoles takes place, but only in the absorbing zone the ions from the medium are taken up into the plasm. By exposing both or one of the zones to the light or by allowing inhibitors to act on these zones, it is possible to obtain valuable data on the processes concerned in uptake and secretion. If for instance an inhibitor on administration to the absorbing zone and to the free zone has a qualitatively different effect on the accumulation in those zones, this must be due to an inhibition of the process which takes the ions from the medium into the plasm, this being the only difference between the absorbing and the free zone.

Analysis of the uptake processes with the aid of light, carbon dioxide and wounding

In 1948 the effect of light was examined. It appeared that exposure to light of the absorbing zone greatly enlarges the accumulation in all leaf zones, also in the absence of CO_2, therefore also without formation of carbo-hydrates. Besides it was shown that exposure of the free leaf part, the absorbing part being in the dark, results in an increased accumulation in the exposed free part. On the one side this points so the influence of the light on the vacuole secretion, on the other tide the increased vacuole secretion at the same time requires a rise in uptake of ions from the medium into the plasm of the absorbing zone, so that the total uptake occurring in this zone, increases considerably as a result of the greater transport to the exposed free leaf part. Especially on the ground of this phenomenon it seemed probable in 1948 that the ions under the influence of an increased concentration gradient would enter the plasm from the medium, so that the concentration in the symplasm, which is low due to the withdrawal of ions, is determining for the quantity of chloride ions taken up from the medium. This gave the impression that the chloride ions can enter the plasm freely.

Besides it was pointed out in 1948 that ion transport takes place in the plasm. After the cutting and wounding of the leaf material to be used for the experiments, the capacity of taking up ions and accumulating them is decreased for some time, but it has already recovered after a few hours. The transport, however, to the free leaf part continues to be considerably weakened for many hours, so that not until 24 hours have passed a normal transport is found. This great sensitiveness indicates that the transport is connected with the plasm. The accumulation in the vacuoles seems to be an irreversible process, the tonoplast almost entirely preventing a leakage of salts from the vacuole under normal circumstances. From redistribution experiments

(Arisz 1954) it has appeared that a slight decrease of accumulated salts may occur, but that under normal conditions these ions do not go to the medium but are transported in the symplasm and thence may be accumulated into the vacuoles or in the cytoplasm.

It is necessary to point out that in less resistant leaves it repeatedly occurs that already on change of the outer solution ions are excreted. It is, however, of essential importance that in resistant material also after a long period of uptake there is no loss of the absorbed chloride ions. Comparatively slight changes in structure of the plasm seem to create the possibility for exosmosis. So in 1957 during some weeks in probably less resistant material under the influence of a treatment in water purified with ion exchangers a great loss of ions was obtained.

On the loss of asparagine various data were already given before (Arisz 1943). As this publication appeared in Dutch in the years of the second world war, we copy a few results rendered in some two figures. Figure 29 gives the result of transfer after uptake for 24 hours in a 1/40 M asparagine solution into a different asparagine solution (8 hours in 1/80 M asp.: C and D; 4 hours in water and next 4 hours in 1/80 M asp. solution: A and B).

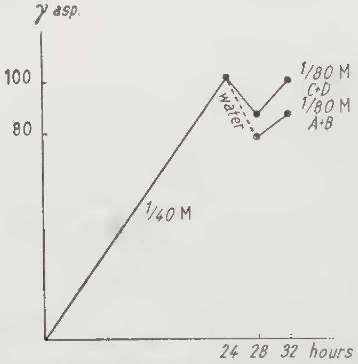


Fig. 29. Absorption of asparagine 0.025 M during 24 hours; afterwards 4 hours in water (A and B) or in 0.0125 M asparagine (C and D). The following 4 hours all series in 0.0125 M asparagine solution.

In fig. 30 the leaves have been taken from a 1/40 M asp. solution to asparagine solutions of various strength after a 24 hours' uptake. Owing to the transfer to a different solution the permeability increases, which is shown as exosmosis or endosmosis dependent upon the concentration gradient between medium and cell sap. After some time the active uptake is resumed. These data indicate that the structure of the plasmatic membranes is not stable and it is influenced by the composition of the medium solution. After some hours an autonomous recovery takes place after which the active uptake is resumed. It may be somewhat upexpected that this lability also holds good for the tonoplast. This, however, must be the case, because Arisz and van Dijk (1939) showed that the asparagine was really taken up into the vacuole and in the exosmosis returns to the medium for the greater part unaltered. This behaviour indicates that the loss of ions is determined by the change in structure of the cytoplasm and that only so long this structure is intact, the normal uptake into and accumulation in the vacuole can take place.

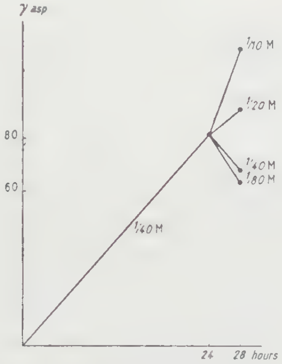


Fig. 30. Absorption of asparagine 0.025 M during 24 hours, afterwards 4 hours in asparagine solutions of different strength.

Analysis of the uptake processes with the aid of inhibitors

In 1953 the influence of inhibitors was investigated. It was ascertained whether an inhibitor administered at the absorbing zone works differently from that in the free part of the leaf. It was found that KCN administered to the absorbing zone can fully inhibit the uptake into the plasm and the accumulation in the vacuoles of the whole leaf. Administered in equal concentration to a zone of the free leaf part, KCN caused no inhibition of accumulation or transport. From this it was concluded that KCN inhibits the uptake into the symplasm and consequently both accumulation and transport to the free part become impossible. The experiments have already been discussed on page 13. Further conclusions inferred from these experiments in 1953 were the following: an ion transport outside the symplasm through the cell walls to the free zones does not occur in a demonstrable degree, because administration of a sufficiently strong concentration KCN to the absorbing zone fully inhibits the transport to the free leaf part and because administration of the same concentration KCN to the free leaf part does not lessen the accumulation. This should be the case if this transport took place through the cell walls outside the symplasm, so that the accumulation in a free zone should be preceded by an uptake from the cell walls.

The behaviour of KCN is the physiological proof of the symplasm theory of the transport, (Arisz 1956).

In 1953 it had likewise appeared from some preliminary experiments that 2,4-dinitrophenol inhibits the accumulation in *Vallisneria* leaves while the transport to other zones and the accumulation in the free leaf part are but slightly decreased by administration to the absorbing leaf part. On continuing these experiments it was regularly found that 2,4-dinitrophenol inhibits the accumulation in the vacuole. Seeing, however, that this inhibitor at a higher concentration can injure the protoplasm, it cannot be said with certainty whether the uptake into the symplasm and the transport in the symplasm continue unaltered. The experiments have not yet been finished, and in the meantime other inhibitors giving a similar effect have been studied. (Cf. fig. 16B).

One of these is *hydroxychinoline* which likewise is slightly injurious to the plasm of less resistant leaves. More favourable were the results with *sodium azide*, which has therefore been extensively examined. The results of the experiments, are briefly summarised here.

Azide on being administered to the middle zone inhibits the accumulation in this zone greatly. On administration to the absorbing leaf part, it likewise inhibits the accumulation whereas the accumulation in the free zones is inhibited to a varying degree, sometimes hardly at all. This accumulation of various strength in the free leaf part indicates that by azide not as by KCN the uptake into the symplasm is inhibited, but the local secretion into the vacuoles and the chloride transport to the free zones.

Azide administered to the absorbing zone usually inhibits the transport through an isolated bundle bridge greatly ("Sutcliffe effect"). Transport by a parenchyma bridge is not inhibited. In the case of a bundle bridge the inhibition of the transport by azide administered to the absorbing part, is probably not based on a direct influence of azide on the transport in the sieve tubes of the bundle, as azide administered to an isolated bundle causes only a slight inhibition of the transport. It might therefore be that azide inhibits the process that takes the salts to the sieve tubes and not the transport itself. This might explain that in experiments with young material this vascular bundle inhibition has not occurred, because then the differentiation of the sieve tubes has not reached the same stage as in older material, so that they still behave as parenchyma cells.

The result of the new experiments with *KCN* was a corroboration of the results obtained in 1953, though the result that KCN does not inhibit the vacuole accumulation in the free leaf part was not always obtained. This effect is apparently influenced by the age of the leaves. In young leaves KCN does not give inhibition of the accumulation, which is proved, when it is administered to a zone of the free leaf part. In older leaves it does give a distinct inhibition of the accumulation. The young material which with KCN did not show inhibition of the vacuole secretion in the free leaf part, did not show it either with arsenate and uranylnitrate. Administered to the absorbing zone, these inhibitors always cause a strong inhibition of the accumulation in all zones of the leaf. This must be due to an inhibition of the uptake into the symplasm. Neither for KCN nor for arsenate and uranylnitrate a difference has been found between bundle bridges and bridges of parenchymatous tissue.

The corroboration of the former observations with KCN on the different reaction of the absorbing and the free zone with two other inhibitors is of great value, because the significance of this fundamental result is considerably increased.

No more can there be any doubt of the former observation with 2,4-dinitrophenol that inhibition of the vacuole secretion may take place without the transport being inhibited, on the ground of the observations with administration of azide to the middle zone, in which the vacuole secretion in this zone is inhibited dependent upon the azide concentration used, while transport to the third zone continues unaltered (figs. 3, 11, 14 and 15). This is independent of the presence of the "Sutcliffe effect". (confer Fig. 21 with arsenate and Fig. 28 with uranyl.)

Nature of the uptake processes

The experiments with KCN, uranylnitrate and arsenate indicate that a process active in the uptake into the symplasm uses an energy-rich phosphate such as adenosinetriphosphate. Uranylnitrate and arsenate both influence the formation of energy rich phosphates, while KCN inhibits the cytochrome oxydase which is active in the production of energy rich phosphates by the mitochondria.

The experiments with inhibitors give the impression that KCN, uranylnitrate and arsenate do not inhibit the accumulation in the vacuole, if the material is still young, but they do, if it is older, whereas dinitrophenol and azide in our experiments always inhibit the vacuole accumulation. Considering our slight biochemical knowledge of the uptake processes, it is difficult to indicate what biochemical reaction this might be based upon. We might think of a surplus of energy rich phosphates present in the plasm in the so-called young, vigorously growing plants, so that inhibition of new formation of these substances cannot influence accumulation. Both for 2,4-dinitrophenol and for azide it is plausible that they do not act on the uptake into the cytoplasm. They always inhibit the accumulation in the vacuole. Even in the young material, in which KCN, uranyl nitrate and arsenate did not inhibit the vacuole secretion, azide did inhibit. In this young material, however, azide also behaved differently, in so far it did not give the specific inhibition of the bundle transport ("Sutcliffe effect"). All these experiments indicate the specific action of these inhibitors on certain biochemical processes.

We shall now revert to the interpretation of the uptake processes. The supposition made in 1948, viz. that chloride ions permeate freely in the plasm so that the vacuole secretion is the only active process in the uptake, had to be abandoned, when it appeared in 1953 that KCN inhibits the uptake into the symplasm. This means that both the uptake into the symplasm and the secretion into the vacuole are connected with metabolism and are processes requiring energy. In 1954 Arisz considered the question whether the process of ions uptake may be active in that sense that anions are passed with difficulty through the plasm with its chiefly negative charge. The ion transfer might be made possible by a reaction that takes away the charge of the ion and may bind it to a carrier, after which the complex can be moved by diffusion or the ions are transferred by an electron ladder system as supposed by Lundegårdh. In a similar system the anions will be present free or bound in the central plasm. If they are free they should be able to interchange with anions in the medium, if there is no outer membrane or if the outer membrane is permeable for anions.

Experiments carried out by the author with Winter (unpublished results) on uptake of labelled chloride ions gave as a result that after uptake there are no labelled chloride ions present in the plasm, which can be exchanged for unlabelled chloride ions in the medium. This means that if the outer membrane should be permeable for anions, no free or exchangeable chloride ions can be present in the plasm. Chloride ions once taken up into the plasm are no more released to the medium and are no more interchangeable with ions in the outer solution either. They are either separated from the medium by a semipermeable membrane impermeable for chloride ions or in case the membrane is permeable for anions they are irreversibly bound to carriers. The lability of the plasmatic structures which we have already mentioned before, particularly in respect of the asparagine uptake cannot elucidate whether the boundary surfaces of the plasm or the carriers themselves, which may be protoplasma molecules (Overstreet) cause the lability.

In 1948 Arisz found a stronger uptake of ions from the medium by a non-exposed absorbing zone when the free part was exposed to light. By the stronger secretion into the vacuoles of the exposed free leaf part ions are withdrawn from the symplasm and this increased the uptake. In 1956 the effect of exposure of the absorbing zone was extensively examined by Arisz and Sol. They found that light increases the uptake into the symplasm, because not only in the absorbing zone but also in the free zones the vacuole secretion greatly increases. So light acts stimulating both on the vacuole secretion and on the uptake into the symplasm. Such a stimulation of the uptake into the symplasm might be explained in various ways. We might consider an increase in permeability of the outer membrane, but this cannot fully explain the light effect, because the uptake is an active process. We may assume that owing to exposure to light more free energy becomes available for the uptake, which can be used for the ion transport direct or indirect. If in the ion transport the ions are bound to carriers the energy must be used either for this binding or for the formation of carriers. Arisz and Sol take it that the stronger uptake after exposure of the free leaf part is due to the formation of substances in the light which are transported to the absorbing zone and are capable of increasing the uptake there. Besides they found that on pre-exposure a transportable substance is formed which in a subsequent exposure is capable of rendering an increased uptake possible for several hours. The nature of these substances is still unknown. They will be called carriers here.

This leads to the question whether in the experiments of this investigation with the effect of inhibitors something has been observed as to inhibition of the formation of these carriers. It might for instance be expected on administration of inhibitors to a middle zone that the accumulation in the absorbing zone would decrease, because now it is not strengthened by the supply of carriers from the middle zone. This is surely not the case for azide. Azide acts quite locally and the accumulation in the absorbing zone has rather increased than decreased in Fig. 3. Neither is there in Fig. 12, 14 and 15 a significant decrease in the accumulation in the absorbing zone.

With uranylnitrate (Figs. 25, 26, 27 and 28) there is always a distinct decrease in accumulation in the absorbing zone, when this inhibitor is administered to the middle zone. The differences, however, are not sufficiently significant to prove that the formation of these carrierlike substances is not inhibited by azide, whereas it is inhibited by uranylnitrate.

The transport of chloride ions

From an experiment on transport in the time (Fig. 1 and Table 2) it appeared that after a rapid accumulation in the absorbing part for the first 3 hours, the accumulation rate in the second period of 3 hours decreases greatly, while from 6–24 hours it takes place at a rate which increases from 14 to 34 μgr. Cl per 20 cms leaflength, 4 mms width per 3 hours. The accumulation in the second and the third zone begins later; it is slow during the first few hours and attains a rate of 23 μgr. Cl in the second 12 hours. From this it appears that it takes a long time before a more distant zone has obtained the full rate of accumulation. The increase in rate indicates an induction process, but it does not yet give an insight in the nature of this process. The formation of carriers mentioned above might bring about such an induction. Apparently a state has been reached after 12 hours, owing to which the accumulation rate in the vacuoles limits the accumulation process. Anyway it is not determined by what has already been taken up into the vacuole.

TABLE 2

Rate of accumulation of chloride in μg/8 leaflengths 2,5 . 0,4 cm/3 hours. The first zone absorbs from a 0,001 M KCl + $CaSO_4$ solution, 25° C.

	1–3 hours	3–6 hours	6–12 hours	12–24 hours
First zone	90	14	21	34
Middle zone	5	14	18	23
End zone	0	5	11	23

In connection with the results communicated before (Arisz and Schreuder) the experiments with bridges have yielded some fresh data. The results are clear and corroborate that a narrow bundle bridge transports almost equally well as the intact tissue. It is a striking fact that the chloride ions are also transported well by parenchyma bridges. This indicates the possibilities of transport by ordinary parenchyma cells. Parenchyma bridges of over 4 mms length appear to transport appreciably less. On the other hand 16 mm bundle bridges still give a proper transport. That is a comprehensible result that indicates a greater suitability for transport of bundle bridges with sieve tubes. In 1913 SOLEREDER mentioned the presence of sieve tubes in these bundles and the lack of xylem vessels.

Particularly surprising is the result of the inhibition of the bundle transport by azide. This removes any doubt one might have that this transport should take place in the plasm. It is also a striking fact that the transported ions even in the parenchyma bridges, which do imply rather drastic injuries, are not released directly to the medium. The results of these experiments are very regular and indicate a diffusion or process of spreading for the transfer of ions in the plasm. About the question whether the ions are transported as such or as complexes with carriers nothing can be said as yet. A connection with metabolism in the transport in the symplasm has not yet been found hitherto. It is, however, possible that we have to deal here with an accelerated diffusion, but experimental data on the rate of transport are still lacking. Vital processes are likely to be involved in the bundle bridges and the transport through the sieve tubes. As discussed on page 24, we see the cause of the inhibition of the bundle transport by azide rather in a process that takes the salts into the sieve tubes than in an inhibition of the transport itself. As already indicated before (ARISZ 1952) the bundles of young leaves will possess sieve tubes which are not yet differentiated. They transport in the same way as ordinary parenchyma cells. There was an indication that in very young leaves the "Sutcliffe effect" with azide does not arise. In this stage apparently the special structure needed for this has not yet been formed.

Distinction of two processes, the uptake into the symplasm and the secretion into the vacuole.

When the uptake processes in *Vallisneria* are compared with other objects the emphasis lies on the possibility *Vallisneria* offers to demonstrate that uptake into the symplasm and accumulation into the vacuole are biochemically different processes.

It is not yet possible to extend this result to other objects like roots. Theoretically it is imaginable that we could prevent the accumulation process in the vacuole by an inhibitor, while the uptake into the symplasm and the secretion into the xylem are continued.

If scientists had not started from the simple diffusion- and permeability phenomena and had not come to the active uptake processes connected with metabolism so gradually, they would probably have distinguished a process of uptake from the medium into the plasm and one of secretion from the plasm into the vacuole from the start. Now that for *Vallisneria* the proof has been yielded for these processes being distinguished, it should be considered whether this distinction is of general validity. It seems of essential importance that it appears from the experiments that the possibility exists of the uptake into the plasm being a process based on a different biochemical reaction from the secretion into the vacuole.

Fig. 31 gives a general scheme of uptake and transport applicable to the root. Uptake from the medium into the central plasm and secretion from the pool of the central plasm into the vacuole are shown as separate processes giving an impression of the possibilities opened up by this constellation. The scheme is based on data obtained with *Vallisneria* leaves. It will be discussed more extensively in other papers. It is based on the assumption of Münch that the cellular protoplasts interconnected by plasmodesms form a symplast in which substances can be translocated. The general presence of plasmodesms has been stressed by Schuhmacher and Lambertz these last few years. The physiological investigations communicated in this and some previous papers have elucidated the role of the symplasm in the plasmatic transport in root and leaf.

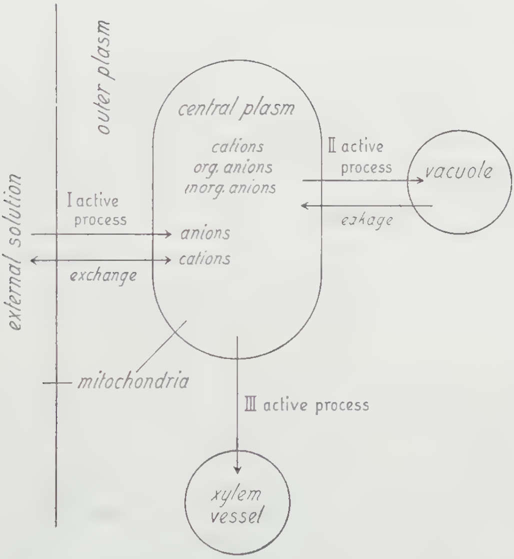


Fig. 31. General scheme of the active processes in the root. The first active process takes the ions into the central plasm. The second active process takes the ions from the central plasm into the vacuole. The third active process takes the ions from the central plasm to the xylem.

In *Vallisneria* leaves beside the active processes of uptake and secretion a migration of salt ions is found from cell to cell in the symplasm. The migrating ions withdrawn from the pool of ions in the central plasm need not be the same ions which are concurrently absorbed from the outer solution.

In the root a third active process seems to be present (Arisz 1945, 1956) which takes the ions from the pool in the central plasm into the xylem vessels (exudation process). The actual mechanism of the active processes is unknown. It suffices that the cation or the anion alone is actively transported for moving both salt ions but it may be that both salt ions are actively transferred. The same problem arises for the three active processes in the root. The ions may be absorbed free or bound to carriers, the bonding may be reversible or irreversible while oxidation reduction processes may be involved in the binding to or the setting free from the carrier.

In the general scheme the representation given is based on the results with *Vallisneria* that in the first active process the anions are transported by an active process from the surface into the central plasm. In the central plasm there are cations which can exchange for cations in the medium.

About the mechanism of the second active process, the accumulation of ions from the pool of the central plasm into the vacuoles, little is known. Presumably ion pairs are translocated. The transport of the cations seems to be the active process. This is suggested by the simultaneous transport of the excess of inorganic cations with organic acid anions from the pool into the vacuole in equivalent amounts.

It is apparent that the ions transported in this accumulation process need not be the same ions which are introduced into the cytoplasm by the concurring first active process. This is valid as well for ions of the same charge but of different kind as for ions and their isotopes. It will be influenced by the number of ions present in the pool of the central plasm in relation to the number of ions translocated in the active processes.

Since cations in the outer solution cannot exchange with cations in the vacuoles, but probably do exchange with cations in the pool of the inner plasm exchange of cations between vacuole and inner plasm is out of the question.

Anions on the other hand which do not exchange between the medium and the pool may possibly exchange between pool and vacuole. This would considerably increase the number of the anions potentially belonging to the pool of the central plasm. An exosmosis of salt from the vacuole or from the central pool to the medium is in this way ruled out.

In several investigations excised roots have been used. This gives an apparent simplification of the system since the effect of the third active process the secretion into the xylem is eliminated. From the general scheme it is apparent that the secretion into the xylem is going on during the absorption, continually removing ions present in the pool of the central plasm. There is no necessity that the same ions which are absorbed are also secreted into the xylem. In studying the accumulation process into the vacuoles the consequence of this change in the composition of the pool in the inner plasm may not be neglected.

A simplification of the general scheme by assuming only one process that takes the ions from outside into the vacuole seems to me to be unwarrented for the present as it excludes all the possibilities mentioned above. If for instance in the central plasm beside the absorbed cations and anions also organic salt ions are present, in the accumulation process an organic anion from the pool can be taken into the vacuole together with a cation, while when taken up from the medium the cation was accompanied by a hydroxyl or bicarbonate ion. It would surely lead to a simpler scheme if the carrier and the nature of the binding of ion to carrier were the same for all active processes, so that the same complex could be carried from the outer layer of the cytoplasm to the vacuoles and to the xylem vessels and from cell to cell over long distances. This supposition, however, is not yet justified by experimental data. It is necessary that for the present we start from a general scheme that leaves the possibility that the carriers and the processes of complex formation can be different, so that the mechanism of the processes can also be of a different nature.

Hitherto this distinction has not been accepted as a matter of fact and it has not been realised that uptake of ions in seaweeds, yeasts and bacteria may behave differently from that in roots, leaves and storage tissue, which contain vacuoles. It is possible that if one is conscious of the fact that the systems may be different and that different biochemical processes are involved, greater agreement can be shown in identical processes.

Acknowledgement

The author has to thank Dr. R. J. Helder for a discussion of the manuscript.

SUMMARY

In this paper the influence of some inhibitors of cell metabolism on the uptake and transport of chloride ions has been investigated.

By comparing the effect of an inhibitor on a part of the leaf where both uptake and accumulation of chloride take place with the effect on an adjoining part where the transported ions are accumulated without uptake from the outer solution data have been obtained about the uptake and the accumulation process separately. Azide inhibits the accumulation in the vacuoles, cyanide, arsenate and uranyl inhibit the uptake from the outer solution.

The transport through tissue bridges of 4 to 16 mm length was estimated and it was found that azide inhibits the transport through a bundle bridge but not through a parenchyma bridge.

The conclusions to be drawn from these results are exposed and a general scheme is given as a basis for the interpretation of the salt transport in *Vallisneria* leaves and plant roots.

REFERENCES

Arisz, W. H. 1943. Versl. Ned. Akad. Wet., Afd. Natuurkunde, *52*: 639–645.
Arisz, W. H. 1945. Proc. Kon. Ned. Akad. Wet. *48*: 420–446.
Arisz, W. H. 1947. Proc. Kon. Ned. Akad. Wet. *50*: 1019–1032.
Arisz, W. H. 1948. Proc. Kon. Ned. Akad. Wet. *51*: 25–32.
Arisz, W. H. 1952. Annual review plant physiol. *3*: 109–130.
Arisz, W. H. 1953. Acta Bot. Neerl. *1*: 506–515.
Arisz, W. H. 1954. Nature *174*: 223.
Arisz, W. H. 1954. Acta Physiol. Pharmacol. Neerl. *3*: 575–599.
Arisz, W. H. 1956. Protoplasma *46*: 5–62.
Arisz, W. H. and M. J. Schreuder. 1956. Proc. Kon. Ned. Akad. Wet., Series C, *59*: 454–461.
Arisz, W. H. and M. J. Schreuder. 1956. Proc. Kon. Ned. Akad. Wet., Series C, *59*: 461–470.

Arisz, W. H. and H. H. Sol. 1956. Acta Bot. Neerl. *5*:218–246.
Butler, G. W. 1954. Physiol. Plant. *6*:617–635.
Cowie, D. B. and R. B. Roberts. 1935. In Electrolytes in Biological Systems, 1–34. (Shanes, A. M., Ed., The American Physiological Society, Washington, D.C., 243 pp., 1955).
Epstein, E. 1955. Plant Physiol. *30*:529–535.
Epstein, E. 1956. Ann. review plant physiol. *7*:1–24.
Hylmö, B. 1953. Physiol. Plant. *6*:333–405.
Hope, A. B. and P. G. Stevens. 1952. Austr. J. Sci. Res. B *5*:335.
Hope, A. B. and P. G. Stevens. 1953. Austr. J. Biol. Sc. *6*:396–409.
Hope, A. B. and R. N. Robertson. 1956. Nature: *177*:43.
Lookeren Campagne, R. N. van. 1956. Proc. Kon. Ned. Akad. Wet., Series C, 59.
Lookeren Campagne, R. N. van. 1957. Acta Bot. Neerl. *6*:543–582.
Lundegårdh, H. 1954. Symp. Soc. exper. Biol. 8.
Lundegårdh, H. 1955. Ann. Rev. Plant Physiol. *6*:1–24.
Overstreet, R. and L. Jacobson. 1952. Ann. review Plant Physiol., *3*:189–206.
Scott, G. T. and H. R. Hayward. 1954. Journ. Gen. Physiol., *37*:601–620.
Solereder, H. 1913. Beitr. Bot. Centr. bl. *30*. *1*:24–104.

Acta Botanica Neerlandica **7** (1958) 33—52

FLORISTISCHE NOTITIES 35–58

S. J. VAN OOSTSTROOM EN TH. J. REICHGELT
(*Rijksherbarium, Leiden*)

(*ingekomen 21 december 1957*)

Deze derde serie Floristische Notities bevat in hoofdzaak aanwinsten van de Nederlandse flora uit de jaren 1955 en 1956. Het merendeel hiervan werd reeds in het kort besproken in de lijsten van nieuwe planten in De Levende Natuur 59, 1956, p. 258–262 en 60, 1957, p. 134–135.

Opmerkelijk is vooral het grote aantal nieuwe adventieven, dat gevonden werd langs de Maas in Limburg, in hoofdzaak bij Meers, gem. Elsloo en tussen Obbicht en Grevenbicht, op terreinen, die werden bezocht tijdens de in 1955 gehouden excursies van het Instituut voor het Vegetatie-onderzoek van Nederland (I.V.O.N.) en van de Commissie voor het Floristisch Onderzoek van Nederland uit de Kon. Ned. Botanische Vereniging (Unio).

Op deze terreinen, stenige en zandige strandjes langs de Maas bij Meers en bij Grevenbicht en grintgroeven tussen Obbicht en Grevenbicht, kwamen behalve de hier besproken nieuwe adventieven nog een groot aantal soorten voor, die reeds eerder in ons land waren aangetroffen. Voor deze verwijzen wij naar de lijst, voorkomende in het Correspondentieblad ten dienste van de floristiek en het vegetatieonderzoek van Nederland no. 1, 1956, p. 8–10. In deze lijst zijn ook een aantal soorten vermeld van soortgelijke terreinen tegenover Maaseyck en bij Ohé en Laak. De zaden en vruchten dezer adventieven zijn wel zeker door de Maas aangevoerd van hogerop in het stroomgebied gelegen fabrieken en losplaatsen; de wolfabrieken aan de Vesdre hebben waarschijnlijk een belangrijk aandeel in deze aanvoer gehad. De gevonden soorten zijn voor een groot deel inheems in het Middellandse Zee-gebied.

No. 52 van deze serie werd bewerkt door Dr A. G. de Wilde, Sassenheim, no. 57 door de heer E. W. Clason, Groningen.

35. **Amaranthus polygonoides** L. (Fig. 1, *a–c*).

Tilburg, terrein Tilburgse Wolwasserij, leg. *J. H. Kern, S. J. van Ooststroom & Th. J. Reichgelt,* 19 sept. 1955, herb. *v. O.* no. 18622, 18626, 18628 (L).

Deze voor de Nederlandse adventief-flora nieuwe soort behoort tot de sectie *Blitopsis* Dum., 1827, en wel tot de groep van deze sectie, waarbij het bloemdek der vrouwelijke bloemen duidelijk vergroeidbladig is. Thellung (1, p. 350) rekent tot deze groep uitsluitend *A. polygonoides* L. Het Nederlandse materiaal behoort tot de subsp. *polygonoides* (subsp. *eu-polygonoides* Thell., l.c. p. 351), die als volgt is te karakteriseren:

Stengel liggend, opstijgend of rechtopstaand, tot 30 cm lang, naar de top kort behaard. Bladen vrij lang gesteeld, de steel iets korter dan tot ongeveer even lang als de schijf; deze ruitvormig tot eirond of omgekeerd eirond, 10–25(–35) mm lang, 3–18 mm breed, aan de top stomp tot uitgerand, met naaldvormig topspitsje, naar de basis geleidelijk versmald. Bloemen in okselstandige kluwens. Schutblaadjes eirond-lancetvormig, priemvormig toegespitst, die der ♀ bloemen ½–⅔ maal zo lang als het bloemdek. Bloemdek der ♀ bloemen 5-tallig, in de vruchttijd ca. 2½ mm lang; de bloemdekbladen in het onderste ⅓ deel sponsachtig verdikt en tot een buis vergroeid, het vrije deel naar buiten gebogen, lijn-spatelvormig, afgeknot tot iets uitgerand, vliezig, met groene, als een kleurloos stekelpuntje uitstekende middennerf en ter weerszijden daarvan met 1 of 2 zwakkere, ± evenwijdige, voor de top eindigende zijnerven. Bloemdek der ♂ bloemen 5-tallig, ca. 1 mm lang; de bloemdekbladen aan de voet nauwelijks vergroeid, lancetvormig, stomp met stekelpuntje of geleidelijk in de spitse punt versmald, vliezig, de 3(–4) buitenste langer en gekield, 1-nervig. Meeldraden 2–3. Vrucht ellipsoidisch of eivormig, ongeveer even lang als het bloemdek en onderaan daarmede samenhangend, bovenaan met een ringvormig verlopende, gerimpelde zoom; top stomp kegelvormig, met 3–4 stijlresten. Zaad in omtrek breed omgekeerd eirond, ca. 1 mm lang, met een vrij scherpe rand, glanzend.

De soort komt voor in de zuidelijke Verenigde Staten, Midden-Amerika en West-Indië. In Europa wordt zij sinds lang in botanische tuinen gekweekt en is zij meermalen daaruit verwilderd; ook in Egypte. Onze plant is zeker met wol aangevoerd.

1. A. Thellung, Amarantus, in Ascherson & Graebner, Synopsis der Mitteleuropäischen Flora 5, 1. Abth., 1914, p. 225–356.

36. **Caltha palustris** L. cf. var. **ranunculiflora** Schur

Mechelen, Limb., moerasje langs de Mecheler beek, leg. *V. Westhoff*, 5 juni 1956 (L).

Op de genoemde plaats werden door Dr. Westhoff planten van *Caltha palustris* aangetroffen, die bloemen hadden van de grootte van die van *Ranunculus acris* L. (bloembladen ca. 6–12 mm lang), doch die overigens geheel met normale planten overeenkwamen. Misschien moeten wij zulke exemplaren tot var. *ranunculiflora* Schur rekenen.

37. **Didesmus bipinnatus** (Desf.) DC. (Fig. 1, *e*).

Meers, gem. Elsloo, Limb., aan de Maasoever, leg. *I.V.O.N.-exc.*, 9 juni 1955, herb. *v. O.* no. 18237 (L); tussen Obbicht en Grevenbicht, grintgroeve aan de Maas, leg. *Unio*, 20 juli 1955, herb. *v. O.* no. 18475 (L).

Het voor de Nederlandse adventief-flora nieuwe Cruciferen-geslacht *Didesmus* Desv., 1814, is nauw verwant met *Rapistrum* Crantz, doch verschilt ervan door het bovenste lid van de hauwtjes, dat ongeveer even dik is als het onderste en tweesnedig of min of meer vierkantig en door de witte bloemkroon. Bij *Rapistrum* is het bovenste lid dikker dan het onderste, niet kantig en op doorsnede cirkelrond (Fig. 1, *d*), terwijl de bloemkroon geel is.

Schulz (1, p. 262) onderscheidt een tweetal soorten, *D. aegyptius*

(L.) Desv. en *D. bipinnatus* (Desf.) DC., in hoofdzaak op de vorm van het onderste lid van het hauwtje. Onze exemplaren behoren tot de laatstgenoemde soort, waarbij dit onderste lid tolvormig en gestreept is; bij *D. aegyptius* is het min of meer kubusvormig en knobbelig. Bij *D. bipinnatus* zijn de bladen meestal dubbel veerdelig met lijnvormige slippen, terwijl ze bij *D. aegyptius* ongedeeld tot liervormig veerdelig zijn, met bredere slippen.

D. bipinnatus komt voor in het westelijke Middellandse Zee-gebied, in Algerië, Tunesië en Lybië.

1. O. E. SCHULZ, Cruciferae-Brassiceae, pars 1. Das Pflanzenreich IV, 105, 1919.

38. **Diplotaxis erucoides** (L.) DC.

Aan de Maas ten N. van Grevenbicht, leg. *I.V.O.N.-exc.*, 8 juni 1955, herb. *v. O.* no. 18214 (L); tussen Obbicht en Grevenbicht, grintgroeve aan de Maas, leg. *Unio*, 20 juli 1955, herb. *v. O.* no. 18474 (L).

Deze *Diplotaxis*-soort is onmiddellijk van de uit Nederland bekende soorten *D. tenuifolia* (L.) DC., *D. muralis* (L.) DC. en *D. viminea* (L.) DC. te onderscheiden door de witte of lichtlila bloemkroon. Zij behoort tot de sectie *Rhynchocarpum* Prantl, gekenmerkt door de boven de kelk niet of zeer kort gesteelde hauwen en de kegelvormige, naar de top versmalde, meestal 1–2-zadige snavel. *D. muralis* en *D. viminea* hebben eveneens boven de kelk niet of zeer kort gesteelde hauwen, doch een smal omgekeerd kegelvormige, naar de voet weinig versmalde of een cylindervormige snavel, zonder zaden. *D. tenuifolia* onderscheidt zich door de boven de kelk duidelijk gesteelde hauwen.

De soort komt door het gehele Middellandse Zee-gebied voor.

39. **Iberis parviflora** Munby (Fig. 1, *f*).

Meers, gem. Elsloo, Limb., aan de Maasoever, leg. *I.V.O.N.-exc.*, 9 juni 1955, herb. *v. O.* no. 18247 (L).

Van deze kleinbloemige *Jberis*-soort — de kroonbladen zijn ten hoogste 3 mm lang — werd één klein, onvertakt exemplaar op bovengenoemde vindplaats aangetroffen. Een korte beschrijving van de soort volgt hier:

Plant eenjarig. Stengel rechtopstaand, ca. 5–15 cm hoog, gewoonlijk vertakt, met zijtakken, die langer zijn dan de hoofdstengel, behaard. Bladen lijn- tot smal omgekeerd lancetvormig, kaal doch aan de rand gewimperd, vrij diep getand, aan weerszijden met 1–3 tanden, de hoogste bladen vaak gaafrandig. Bloeiwijze, ook in de vruchttijd, kort, scherm-trosvormig. Bloemen klein; kelkbladen groen, vaak rood aangelopen, ca. 2 mm lang; kroonbladen wit (of rose), ca. 3 mm lang. Vrucht ca. 6–8 mm lang, ongeveer even lang als breed, rondom gevleugeld; vleugelrand aan de top uitlopend in twee driehoekige, spitse lobben, die een stompe hoek met elkaar maken; stijl ongeveer tot de helft van de lengte der insnijding reikend.

Voor zover ons bekend is, werd deze soort tot nu toe alleen in Algerië gevonden.

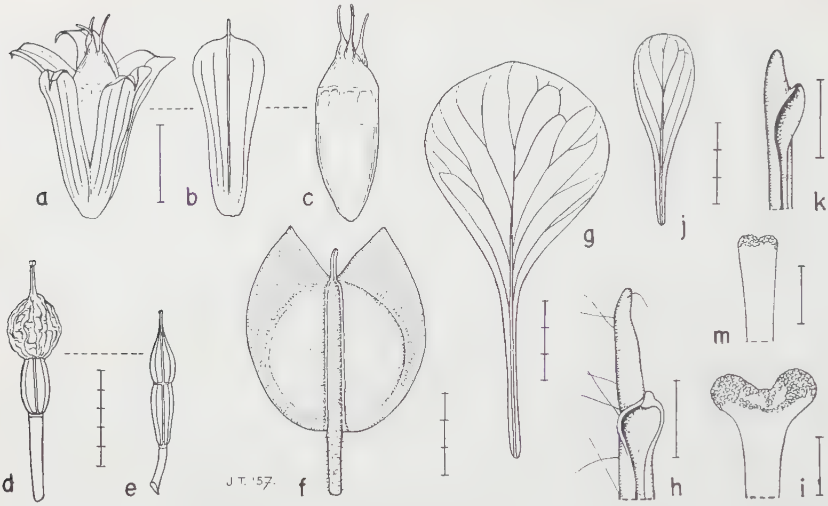


Fig. 1. *a–c: Amaranthus polygonoides* L., *a:* bloemdek met vrucht, *b:* bloemdekblad, *c:* vrucht; *d: Rapistrum rugosum* L., vrucht; *e: Didesmus bipinnatus* (Desf.) DC., vrucht; *f: Iberis parviflora* Munby, vrucht; *g–i: Sisymbrium septulatum* DC., *g:* kroonblad, *h:* top van een buitenst kelkblad, *i:* stempel; *j–m: Sisymbrium altissimum* L., *j:* kroonblad, *k:* top van een buitenst kelkblad, *m:* stempel. Schaal in mm.

40. **Lepidium draba** L. subsp. **chalepense** (L.) Thell. var. **auriculatum** (Boiss.) Thell.

Tussen Obbicht en Grevenbicht, grintgroeve aan de Maas, leg. *Unio*, 20 juli 1955, herb. *v. O.* no. 18447 (L).

Van *Lepidium draba* L. onderscheidt Thellung (2, p. 85 en 86) een tweetal ondersoorten, subsp. *eu-draba* Thell., l.c. p. 85 (= subsp. *draba*) en subsp. *chalepense* (L.) Thell., l.c. p. 86; de eerste heeft hauwtjes, die aan de basis zwak hartvormig zijn, bij de tweede zijn ze aan de voet afgeknot, afgerond of versmald.

Tot nu toe was van subsp. *chalepense* uit Nederland alleen bekend de var. *typicum* Thell., l.c. p. 86 (= var. *chalepense*) met omgekeerd eironde tot elliptisch-langwerpige of eironde, vaak iets boven de basis versmalde bladen. Zie Henrard (1, p. 219). Het boven geciteerde exemplaar heeft echter lancetvormige tot smal lancetvormige, boven de basis niet versmalde bladen en behoort tot var. *auriculatum* (Boiss.) Thell., l.c. p. 86; deze variëteit is meestal geheel kaal; onze plant is echter behaard en moet tot f. *canescens* Thell., l.c. p. 86 worden gerekend.

1. J. Th. Henrard, Lepidium. Ned. Kruidk. Arch. 1916, p. 206–247.
2. A. Thellung, Die Gattung Lepidium (L.) R.Br. Mitt. Bot. Mus. Univ. Zürich 28, 1906.

41. **Matthiola** cf. **oxyceras** DC.

Tussen Obbicht en Grevenbicht, grintgroeve aan de Maas, leg. *Unio*, 20 juli 1955, herb. *v. O.* no. 18458 en 18470 (L).

De gevonden planten, een tweetal, behoren waarschijnlijk tot deze soort. Zekerheid hebben wij echter niet, daar de exemplaren alleen zeer jonge hauwen dragen en voor een juiste determinatie rijpe vruchten beslist nodig zijn. Waarschijnlijk behoort ook een door Dr. W. Feekes in aug. 1941 op Schokland verzamelde en door KLOOS (1, p. 39) als *M. tricuspidata* (L.) R.Br. beschouwde plant tot deze soort.

1. A. W. KLOOS Jr., Aanwinsten van de Nederlandse flora in 1942. Ned. Kruidk. Arch. 53, 1943, p. 30–45.

42. **Sisymbrium septulatum** DC. (Fig. 1, *g–i*).

Tussen Obbicht en Grevenbicht, grintgroeve aan de Maas, leg. *I.V.O.N.-exc.*, 7 juni 1955, herb. *v. O.* no. 18197 (L; ook in herb. De Jongh).

Deze opvallend grootbloemige *Sisymbrium* behoort met de in ons land vrij veel adventief voorkomende of zelfs ingeburgerde *S. altissimum* L. en *S. orientale* L. tot de sectie *Pachypodium* (Webb & Berth.) Fourn.

De bij ons gevonden planten — er werden op de genoemde plaats drie exemplaren aangetroffen — behoren tot de typische ondersoort, subsp. *septulatum*. Naast deze onderscheidt SCHULZ (1, p. 371, 372; 2, p. 121, 122) een tweetal geografische rassen, die o.i. ook beter als subspecies kunnen worden opgevat, nl. subsp. *rigidulum* (Decaisne) nob. (*S. rigidulum* Decaisne in Ann. Sc. Nat. Bot. 2. sér., 3, 1835, p. 272) en subsp. *bilobum* (C. Koch) nob. (*Diplotaxis biloba* C. Koch in Linnaea 15, 1841, p. 252).

De subsp. *septulatum* verschilt van *S. altissimum* L., waarmede ze habitueel en in bladvorm veel gelijkenis vertoont, al op het eerste gezicht door de grote bloemen, met kelkbladen van 6–9 mm en kroonbladen van ca. 15 mm lengte. De twee buitenste kelkbladen zijn aan de top duidelijk kapvormig, met een tot ca. 1½ mm lang topspitsje; de plaat van de kroonbladen is breed omgekeerd eirond en gaat plotseling in de smal lijnvormige nagel over; de stempel is diep 2-lobbig; verder steken in de tros de bloemknoppen ver boven de bloemen uit.

Bij *S. altissimum* (Fig. 1, *j–m*) zijn de kelkbladen 4–6 mm lang en de kroonbladen 6–8 mm; het topspitsje van de kelkbladen is korter, nog geen ½ mm lang; de plaat der kroonbladen is smaller en gaat geleidelijk in de nagel over; de stempel is kort 2-lobbig; bovendien staan in de tros de bloemen ongeveer op gelijke hoogte met de knoppen.

De soort komt voor in de landen ten oosten van de Middellandse Zee, van Syrië tot Afghanistan; de subsp. *septulatum* is gevonden in Syrië, Mesopotamië, Koerdistan, Perzië en Afghanistan.

1. O. E. SCHULZ, Sisymbrium septulatum DC., eine bisher nicht genügend bekannte Art. Fedde, Repert. 15, no. 24, 1918, p. 369–372.
2. O. E. SCHULZ, Cruciferae-Sisymbrieae. Das Pflanzenreich IV, 105, 1924.

43. **Hippocrepis unisiliquosa** L. (Fig 2, *b*).

Meers, gem. Elsloo, Limb., aan de Maasoever, leg. *Unio*, 21 juli 1955, herb. *v. O.* no. 18536 (L).

Van het geslacht *Hippocrepis* L. was tot nu toe slechts één soort, *H. comosa* L., uit ons land bekend, en wel van een uiterwaard aan de Lek tussen Ameide en Lexmond, waar ze in groot aantal en zeer waarschijnlijk indigeen voorkomt; voorts van de Staart op het eiland van Dordrecht, en daar mogelijk adventief.

Een tweede soort, *H. unisiliquosa* L., werd in slechts één exemplaar aangetroffen aan de Maasoever bij Meers. De voornaamste kenmerken ervan zijn de volgende:

Plant eenjarig. Bloemen meestal alleenstaand in de bladoksels, zelden 2 of 3 bijeen, bijna zittend. Peul recht tot gebogen, 3-5 mm breed, kaal of aan de rand van de leden, zelden geheel, ruw door grote papillen.

Hrabětová-Uhrová (1, p. 143, 144) verdeelt de soort in een tweetal ondersoorten, nl. subsp. *eu-unisiliquosa* Hrab. (= subsp. *unisiliquosa*) en subsp. *bisiliqua* (Forsk.) Bornm., te onderscheiden als volgt:

Subsp. *unisiliquosa*: Bloemen alleenstaand, zelden 2 of 3 bijeen. Peul ongeveer recht of ± gebogen, 3–5 mm breed, meestal kaal, de openingen van de leden naar de convexe zijde van de peul gericht.

Subsp. *bisiliqua* (Forsk.) Bornm.: Bloemen 2, zelden 3 bijeen, soms alleenstaand. Peul vaak gebogen, 3–4 mm breed, geheel of aan de rand van de leden door papillen ruw, de openingen van de leden naar de concave zijde van de peul gericht.

Het bij ons gevonden exemplaar behoort tot de laatste ondersoort. Deze komt voor in Transcaucasië, Perzië, Mesopotamië, Arabië, Syrië, Palestina en Egypte, terwijl de typische ondersoort in het gehele Middellandse Zee-gebied gevonden wordt, oostelijk tot Perzië.

1. A. Hrabětová-Uhrová, Generis Hippocrepis L. revisio IV. Acta Acad. Sci. Nat. Moravo-Siles. 22, 4, 1950, p. 99-158.

44. **Psoralea americana** L. (*P. dentata* DC.). (Fig. 2, *a*).

Middelburg, Segeersweg, leg. *C. Brakman*, 16 nov. 1942 (L); Soest, vuilstortterrein, leg. *R. Tolman*, 3 nov. 1953 (L).

Deze adventieve soort van het *Papilionaceae*-geslacht *Psoralea* L., waarvan tot nu toe nog geen vertegenwoordiger in Nederland was aangetroffen, is als volgt gekenmerkt:

Plant kruidachtig, overblijvend. Stengels rechtopstaand of opstijgend, ca. 40–100 cm lang, vrij dicht behaard en, evenals de rest van de plant, dicht met zittende klieren bezet. Bladen 3-tallig, met kort gesteelde zijblaadjes en lang gesteeld topblaadje; blaadjes ruitvormig eirond, elliptisch of omgekeerd eirond, ca. 2–5 cm lang en 1½–3½ cm breed, aan de voet gaafrandig, overigens verwijderd gegolfd-getand. Bloeiwijze okselstandig, lang gesteeld, veelbloemig, bestaande uit trosvormig gerangschikte bloemgroepjes, ongeveer even lang als of duidelijk langer dan het blad. Bloemen in de oksel van lancetvormige, blijvende schutblaadjes; kelk met ongelijke, lancetvormige tanden, beklierd en bijna kaal of dicht behaard; bloemkroon ca. 8 mm lang, wit- of paarsachtig, de kiel met donkere vlek. Peul ongesnaveld, eenzadig, niet openspringend, door de kelk ingesloten.

De soort komt voor in westelijk Noord-Afrika (Marokko, Algerië, Tunesië), op Madeira en in Spanje en Portugal; zij is verder adventief aangetroffen in Zwitserland (1, p. 1385) en Engeland (2, p. 37).

Onze plant behoort door de dicht behaarde en beklierde kelk tot var. *villosa* (Guss.) Thell. (*P. polystachya* Poir.).

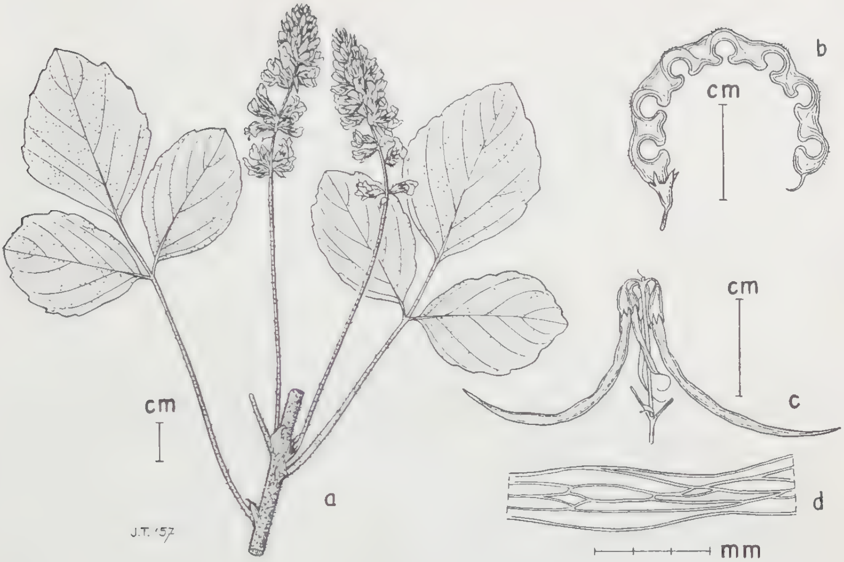


Fig. 2. *a: Psoralea americana* L., deel van een bloeiende tak; *b: Hippocrepis unisiliquosa* L., peul; *c–d: Trigonella caelesyriaca* Boiss., *c:* peulen, *d:* deel van een peul, sterker vergroot.

P. americana L. is van alle tot nu toe hier te lande aangetroffen Leguminosae onmiddellijk te onderscheiden door de dichte bekliering met grote, donkere, zittende klieren.

1. H. Gams, Leguminosae in Hegi, Illustr. Fl. Mitt. Europa IV, 3, 1923.
2. D. H. Kent, Plant Notes. Proc. Bot. Soc. Brit. Isl. 1, 1954.

45. **Trifolium cernuum** Brot.

Tilburg, bij een wolfabriek, leg. *S. E. de Jongh, S. J. van Ooststroom & Th. J. Reichgelt,* 19 sept. 1953, herb. *Kern & Reichgelt* no. 13199 (L).

Een enkel, zeer klein exemplaar van deze eenjarige, rose bloeiende klaver werd op genoemde vindplaats aangetroffen. De soort is nauw verwant met de reeds eerder bij ons adventief gevonden *T. parviflorum* Ehrh. Zij verschilt van deze laatste o.a. door de bochtige, slanke stelen der hoofdjes, de bloemsteeltjes, die ongeveer even lang zijn als de kelkbuis en reeds tijdens de bloei terugbuigen, de vrij losbloemige hoofdjes, de weinig ongelijke kelktanden en de uitgerande vlag. *T. parviflorum* heeft dikkere, stijvere hoofdjesstelen, bloemsteeltjes, die ½–⅓ maal zo lang zijn als de kelkbuis en pas in de vruchttijd terugkrommen, dichtbloemige hoofdjes, duidelijk ongelijke kelktanden en een vrij spitse of afgeronde, niet uitgerande vlag.

T. cernuum is inheems in Zuid-Frankrijk, op Corsica en in Spanje en Portugal.

46. **Trifolium rubens** L.

Rotterdam, handelsterrein aan de Rosestraat, leg. *P. Jansen & W. H. Wachter* no. 6063–6064, juni 1905 (L).

In het Rijksherbarium vonden wij de hierboven opgegeven adventieve exemplaren van *T. rubens*, een soort, die nog niet eerder in de Nederlandse literatuur werd vermeld, hoewel zij reeds in 1905 werd gevonden.

Van de in Nederland voorkomende *Trifolium*-soorten staan *T. pratense* L. en *T. medium* L. het dichtst bij *T. rubens;* deze verschilt van de beide andere in hoofdzaak door het volgende:

Plant grotendeels kaal, alleen de kelktanden lang gewimperd. Blaadjes langwerpig- tot lijn-lancetvormig, duidelijk fijn en scherp getand. Steunblaadjes langer dan de bladsteel, geheel kruidachtig. Hoofdjes lang ei- tot cylindervormig.

T. rubens L. komt voor in Midden- en Zuid-Europa.

47. **Trigonella caelesyriaca** Boiss. (Fig. 2, *c–d*).

Meers, gem. Elsloo, Limb., aan de Maasoever, leg. *I.V.O.N.-exc.*, 9 juni 1955, herb. *v. O.* no. 18249 (L); id., leg. *Unio*, 21 juli 1955, herb. *v. O.* no. 18497 (L); id., leg. *S. J. van Ooststroom & Th. J. Reichgelt* no. 18668, 23 sept. 1955 (L).

Hoewel ons materiaal niet geheel klopt met de door Širjaev (1, p. 19) gegeven beschrijving, twijfelen wij er niet aan, dat het tot *T. caelesyriaca* behoort, temeer daar het volkomen identiek is met een door Širjaev geciteerd nummer (*Meyers & Dinsmore* G. 1760), dat zich in het Rijksherbarium bevindt.

Een korte beschrijving volgt hier:

Plant eenjarig. Stengels liggend, opstijgend of rechtopstaand, tot 30 cm lang, soms nog langer, evenals de rest van de plant kaal of met enkele verspreide haren bezet. Blaadjes breed driehoekig-omgekeerd eirond tot soms smal omgekeerd eirond, aan de wigvormige voet gaafrandig, aan de breed afgeronde, afgeknotte of iets uitgerande top getand, met brede, vrij stompe tot vrij spitse tanden. Steunblaadjes eirond-lancetvormig, bij hun voet meer of minder diep getand of geheel gaafrandig. Bloeiwijze 6–12-bloemig, vrijwel schermvormig, de steel ongeveer even lang als het erbij behorende blad; bloemsteeltjes ongeveer even lang als de kelkbuis, tenslotte teruggeslagen (volgens Širjaev na de bloei teruggeslagen, doch later weer opgericht of horizontaal uitstaand, wat bij onze planten en ook bij het bovengenoemde exemplaar van Meyers & Dinsmore niet het geval is). Kelk ca. 3 mm lang; de buis klokvormig, de tanden bijna gelijk, smal driehoekig, spits, ± ⅓ maal zo lang als de buis. Bloemkroon geel, ca. 7 mm lang; de vlag aan de top breed en diep uitgerand, iets langer dan de zwaarden, deze iets langer dan de kiel. Peul ongeveer rolrond, 3–4,5 cm lang, ca. 2 mm breed, recht tot halfcirkelvormig gebogen, aan de top geleidelijk versmald; nerven in de lengterichting iets scheef verlopend, hier en daar anastomoserend. Zaden in omtrek langwerpig, met stompe uiteinden, 3 × ca. 1½ mm, fijn geknobbeld.

De soort komt voor in het gebied direct ten oosten van de Middellandse Zee, in Palestina, Syrië, Armenië en Mesopotamië.

1. G. Širjaev, Generis Trigonella L. revisio critica II. Publ. Fac. Sci. Univ. Masaryk 110, 1929, p. 3–37.

48. **Oenanthe aquatica** (L.) Poir.

De heer R. Tolman te Soest maakte ons opmerkzaam op het voorkomen van exemplaren van deze soort met opvallend purperkleurige bloemen op een aantal plaatsen aan of bij de kust van het IJselmeer (Muiden, Eemnes, Spakenburg, Nijkerk, Putten). Hij nam deze reeds enige jaren waar en meent te kunnen constateren, dat het verschijnsel alleen bij laatbloeiende planten optreedt; hij spreekt zelfs van een herfst- en wintervorm. Opmerkelijk is, dat voor zover ons bekend, in de literatuur uitsluitend witbloeiende *O. aquatica* wordt vermeld.

49. **Pimpinella puberula** (DC.) Boiss.

Tussen Obbicht en Grevenbicht, grintgroeve aan de Maas, leg. *Unio*, 20 juli 1955, herb. *v. O.* no. 18454 (L).

In tegenstelling tot de twee inheemse soorten van het geslacht *Pimpinella*, *P. major* (L.) Huds. en *P. saxifraga* L., die beide overblijvend zijn en kale vruchten bezitten, is *P. puberula* eenjarig en heeft behaarde vruchten. Wat deze kenmerken betreft komt de soort overeen met de gekweekte en soms verwilderde of adventieve *P. anisum* L. Zij verschilt hiervan o.a. door de smal lijn- tot draadvormige slippen der bovenste bladen en door de vrij lang en afstaand behaarde vruchtbeginsels. Bij *P. anisum* zijn de genoemde bladslippen breder, lijn- tot lijnlancetvormig, terwijl de vruchtbeginsels korter- en aangedrukt behaard zijn.

P. puberula (DC.) Boiss. komt voor in het oostelijke Middellandse Zee-gebied, oostelijk tot in Perzië.

50. **Cuscuta australis** R.Br. (Fig. 3, *a–b*).

Oostelijk Flevoland, perceel R, opslagplaats van Koreaanse matten, op *Polygonum bungeanum* Turcz., leg. *J. Diender*, 11 aug. 1952, herb. *IJselmeerpolders* no. 957 (L).

Cuscuta australis R.Br. behoort evenals de reeds eerder in Nederland gevonden *C. campestris* Yuncker, *C. gronovii* Willd. en *C. suaveolens* Ser. tot het subgenus *Grammica* (Lour.) Yuncker, sectie *Clistogrammica* Engelm. em. Yuncker. Zij kan op de volgende wijze in de door VAN OOSTSTROOM (3, p. 163) gegeven determinatie-tabel worden ingevoegd:

3a. Bloemkroon kort en wijd klokvormig, de kroonbuis iets korter tot iets langer dan de slippen 4

b. Bloemkroon buis-klokvormig, de kroonbuis 1½ tot bijna 3 maal zo lang als de slippen 5

4a. Kroonslippen spits, breed driehoekig tot eirond driehoekig met vrijwel rechte of weinig convexe randen. Kelkslippen aan de voet met de randen iets over elkaar heen liggend, breed eirond tot cirkelrond of zelfs meer breed dan lang, stomp tot breed afgerond aan de top. Kroonschubben ongeveer even lang als de kroonbuis, langwerpig tot langwerpig-eirond, niet tweespletig, met talrijke wimpers . . . . **C. campestris**

b. Kroonslippen vrij stomp tot stomp, eirond tot breed eirond, met duidelijk convexe randen. Kelkslippen aan de voet niet met de randen over elkaar heen liggend, eirond tot breed eirond met stompe top. Kroonschubben duidelijk korter dan de kroonbuis, tweespletig, met weinig wimpers. . . . . **C. australis**

5, zie 4 in de oorspronkelijke tabel: *C. gronovii* en *C. suaveolens*, enz.

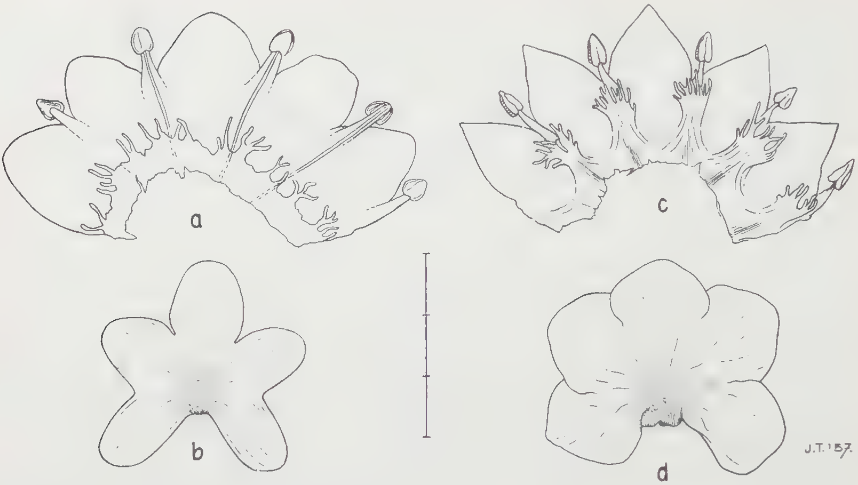


Fig. 3. *a–b: Cuscuta australis* R.Br., *a:* bloemkroon, *b:* kelk, beide opengeslagen; *c–d: C. campestris* Yuncker, *c:* bloemkroon, *d:* kelk, beide opengeslagen. Schaal in mm.

HJELMQVIST (2) beweert, dat de meeste voor Nederland als *C. campestris* vermelde exemplaren niet tot deze soort, doch tot *C. australis* R.Br. var. *cesatiana* (Bert.) Yuncker behoren. Een deel van het door hem als zodanig beschouwde Zweedse materiaal zou namelijk met tuinzaden uit Nederland in Zweden zijn ingevoerd. Uit de door Hjelmqvist gegeven uiteenzettingen en uit zijn afbeeldingen menen wij op te maken, dat hij twee, een weinig verschillende vormen van *C. campestris* op het oog heeft, de ene met kroonslippen, die vrijwel rechte randen hebben, de andere met iets convexe randen der slippen; bij de eerste zijn deze bovendien geleidelijk naar de spitse top versmald, bij de andere vrij plotseling toegespitst. Een dergelijk verschil, dat o.i. geheel binnen de variabiliteit van *C. campestris* valt, wordt bij de Nederlandse planten ook aangetroffen. De o.i. voor *C. campestris* (Fig. 3, *c–d*) karakteristieke kenmerken, nl. de spitse kroonslippen, die vaak een ingebogen topje hebben, de vorm en grootte van de kroonschubben en ook de aan de voet met de randen iets over elkaar heen grijpende kelkslippen, laten geen twijfel, dat alle Nederlandse als *C. campestris* beschouwde exemplaren, ook de vroeger in de Nederlandse literatuur als *C. australis* var. *cesatiana* vermelde, werkelijk tot *C. campestris* behoren. De voor *C. australis* karakteristieke kenmerken, nl. de stompe of vrij stompe, aan hun randen duidelijk convexe kroonslippen, zonder naar binnen gebogen topje, de meestal 2-delige, weinig gewimperde kroonschubben, die gewoonlijk korter zijn dan de kroonbuis en de aan de voet met de randen niet over elkaar heen grijpende kelkslippen ontbreken bij de Nederlandse planten geheel. O.i. is dit ook het geval met het door Hjelmqvist afgebeelde Zweedse materiaal.

Voor de verschillen tussen *C. campestris* en *C. australis* var. *cesatiana* verwijzen wij ook naar de instructieve figuren van CAMPANILE (1, p. 362 en 363, fig. 3, 4 en 5).

1. G. CAMPANILE, Sopra alcune specie di Cuscuta della sezione Clistogrammica. Annali di Botanica 16, 1926, p. 357–379.
2. H. HJELMQVIST, Cuscuta australis R.Br. i Sverige. Bot. Notiser 1953, p. 97–104.
3. S. J. VAN OOSTSTROOM, Het geslacht Cuscuta in Nederland. Ned. Kruidk. Arch. 52, 1942, p. 159–210.

51. **Gilia multicaulis** Benth. var. **peduncularis** (Eastw.) Jepson

Noordoostpolder, zandafgraving bij De Voorst, leg. *J. Diender*, 4 sept. 1956, herb. *IJselmeerpolders* no. 3408 (L).

Enige soorten van het geslacht *Gilia* worden bij ons als sierplant gekweekt; *G. multicaulis* echter wordt niet in de Nederlandse tuinbouwliteratuur vermeld en is zeer waarschijnlijk als adventief te beschouwen. De boven vermelde variëteit is, evenals de typische vorm van de soort afkomstig uit Zuid-Californië.

De soort is als volgt te karakteriseren:

Plant eenjarig. Stengels rechtopstaand of opstijgend, onvertakt tot sterk vertakt, 15–50 cm lang. Bladen enkel- tot dubbel veerdelig, met lijn- tot draadvormige slippen, de onderste gesteeld, de bovenste zittend en minder gedeeld tot ongedeeld, kaal of zwak behaard, niet of zeer zelden beklierd. Bloemen in 2–7-bloemige, lang gesteelde kluwens, soms alleenstaand. Kelk tot op ⅓–½ van zijn lengte ingesneden, de kelktanden driehoekig-lancetvormig tot lancetvormig, toegespitst, met vliezige, vaak paars gekleurde verbindingsstroken. Bloemkroon trechtervormig, ca. 6–10 mm lang, ongeveer 2 maal zo lang als de kelk, met geelachtige buis en witachtige, blauwe of rose slippen. Meeldraden ingeplant bij de bochten van de kroon en korter dan de kroonslippen. Doosvrucht eivormig-langwerpig, met veelzadige hokken.

De var. *peduncularis* (Eastw.) Jepson wijkt in hoofdzaak af door de bladen met weinige, ver uiteenstaande slippen en de gewoonlijk alleenstaande bloemen op ca. 2 tot 6 cm lange stelen.

52. **Euphrasia borealis** Townsend (door A. G. DE WILDE). (Fig. 4, *a–b*).

Toen KLOOS (1) zijn verhandeling over het genus *Euphrasia* in Nederland publiceerde, had hij deze studie geheel gebaseerd op de monografie van WETTSTEIN (4). Hoewel voor de Nederlandse floristiek de publicatie van Kloos bijzonder belangrijk is, bleek toch telkens weer, dat exemplaren werden gevonden die niet met zekerheid op naam waren te brengen, wat bij dit kritische geslacht ook wel te verwachten was.

De meeste soorten, die men tot op heden in de Nederlandse flora's vermeld vindt, hebben hun verspreidingsgebied voornamelijk in Midden- en Zuid-Europa. De ligging van Nederland echter, aan de zuidgrens van het areaal van boreaal-atlantische soorten, doet vermoeden dat hier ook meer van deze noordelijke soorten dan alleen *E. littoralis*, *suecica* en *parviflora* te vinden zullen zijn. Bovendien bestaat de mogelijkheid belangwekkende tussenvormen van continentale of mediterrane en boreaal-atlantische soorten aan te treffen. Tijdens een N.J.N.-zomerkamp op Schiermonnikoog in 1952 werden door mij enkele opvallend forse *Euphrasia*-exemplaren verzameld. Later bleek dat deze tot *E. borealis* zouden kunnen behoren. Dit vermoeden werd tijdens de zomerexcursie van de K.N.B.V. van 1956 bevestigd.

De beschrijving van *Euphrasia borealis* Townsend luidt als volgt:

Forse, donker bebladerde planten met voorzomerhabitus, (10–)15–30(–35) cm hoog. Stengel vaak wat bochtig, rechtopstaand of opstijgend, dik en vaak stijf, groenachtig tot roodbruin, dicht met korte, witte, teruggekromde haren bezet, meestal weinig vertakt; vertakking onder of omstreeks het midden van de stengel; takken meestal paarsgewijs, korter dan de hoofdstengel, opgericht. Bladen en schutbladen schuin tot horizontaal afstaand, donkergroen, zonder sterk uitspringende nerven aan de onderzijde. Bladen veel korter dan de internodiën, eirond of elliptisch, met korte, wigvormige voet, kaal of met kleine borstels aan de rand; de onderste stomp, met 1 tot 2 paar stompe tanden en brede eindtand met ongeveer evenwijdige zijranden, kort behaard op de nerven aan de onderzijde en verspreid behaard aan de bovenzijde; de hogere met min of meer spitse onderste tanden en in vorm geleidelijk overgaand in de onderste schutbladen. Schutbladen kaal of bijna kaal aan boven- en onderzijde, de onderste korter, de bovenste langer dan de internodiën; de onderste breed eirond met hartvormige tot afgeronde voet en met spitse onderste en naar de bladtop stompere tanden; middelste en bovenste schutbladen breed ruitvormig tot eirond met versmalde voet en 3–5 spitse, soms genaalde tanden. Bloemen vanaf de (5–)8(–11)^{e} knoop. Kelk licht grijsgroen, kaal of met korte borstels op de donkergroene nerven, met donkergroene, smal driehoekige, tot 3 mm lange, spitse, soms genaalde tanden; in de vruchttijd sterk vergroot, tot 8 mm lang en 2,5 mm breed. Kroon gewoonlijk wit met blauwachtige bovenlip; onderlip met gele vlek en donkerbruine-paarse aderen, 6–8 mm lang, iets langer dan de bovenlip, met brede, uitgerande middenslip, die langer is dan de brede, uitgerande zijslippen. Doosvrucht groot, ca. 6,5 mm lang en 2,5 mm breed, meestal korter dan de kelk, langwerpig, aan de top afgerond en iets uitgerand, duidelijk gesteeld (steel 1–1,5 mm), dun verspreid behaard tot kaal, aan de rand lang gewimperd. Zaden lichtbruin, tot 2 mm lang. Bloeitijd: juli–augustus.

Verspreiding in Nederland: Bekend van Schiermonnikoog (*Den Hartog & De Wilde*, 1952), later tijdens de Unio-1956 daar opnieuw verzameld. Komt vooral voor in de met kruipwilg begroeide, wat vochtige duinterreintjes. Het materiaal bevindt zich in herb. *De Wilde* en in L.

Verspreiding buiten Nederland: Fär Öer, Schotland, Noord-Engeland, Ierland, Denemarken, kustgebied van Noorwegen.

De beschrijving door Wettstein (4) past zeer goed op het Nederlandse materiaal, alleen is zijn opgave van de kroonlengte (ca. 8 mm) voor onze planten aan de hoge kant. De afbeelding in het artikel van Pugsley (2) toont exemplaren die de indruk maken identiek te zijn

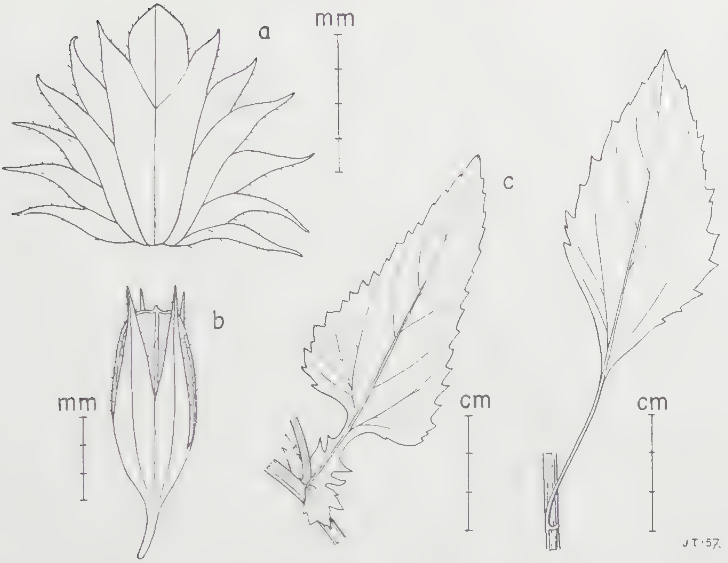


Fig. 4. *a–b: Euphrasia borealis* Townsend, *a:* schutblad, *b:* kelk met doosvrucht; *c:* blad van *Verbesina encelioides* (Cav.) Benth. & Hook. var. *encelioides; d:* blad van *V. encelioides* (Cav.) Benth. & Hook. var. *exauriculata* Robins. & Greenm.

met de planten van Schiermonnikoog. In het Rijksherbarium bevinden zich van *E. borealis* alleen enkele exemplaren van Dyke Hills, Brighton, sept. 1900, coll. *Hilton.* Blijkens het etiket was de determinatie niet zeker, maar het materiaal komt vrij goed met het onze overeen.

Hoewel *E. borealis* door zijn forse habitus en donkere bebladering zeer opvallend is, zijn de grenzen met andere soorten niet altijd gemakkelijk vast te stellen. Zo zou in Nederland *E. brevipila* Burnat & Gremli kunnen voorkomen. Deze soort is verspreid over Noord-, Midden- en West-Europa (Pugsley geeft op Schotland, Ierland, Engeland, IJsland, Scandinavië tot in West-Rusland en West-Pruisen en in de Franse, Zwitserse en Oostenrijkse Alpen; Newfoundland en Quebec). Hoewel voornamelijk in berggebieden voorkomend, is zij niet daartoe beperkt. Het onderscheid der beide soorten ligt hoofdzakelijk in het feit dat *E. brevipila* korte klierharen bezit, tenminste op de randen en bases der schutbladen en een lichtgroene bladkleur heeft, maar er komt ook een klierloze vorm voor (f. *sub-eglandulosa* Bucknall). Bij het Nederlandse *borealis*-materiaal heb ik bij enkele exemplaren (weinige) korte klierharen aangetroffen! Townsend (3) schrijft in zijn monografie der Engelse *Euphrasia*-soorten: "Future observation may possibly determine that *E. borealis* is an eglandular form of *E. brevipila* which has a separate area, and a more northern extension than the parent form, and that this eglandular form is constant within this area except on its southern border." Hoewel ik me met deze opvatting niet kan verenigen (*E. brevipila*-materiaal in het Rijksherbarium is duidelijk anders dan *E. borealis*) komen beslist tussenvormen voor.

Ook de grens tussen *E. borealis* en *E. stricta* is niet scherp. Van *E. borealis* komt voor de var. *zetlandica* Pugsley, die kleiner is (tot 20 cm), kortere internodiën heeft, scherper getande schutbladen. langere kroon (8–9 mm), die vaak paars is, een doosvrucht die langer is dan de kelk, welke minder scherpe tanden heeft. Dit zijn *borealis*-vormen die naar het *stricta*-type neigen. Bij de Schiermonnikoogse planten heb ik ze (nog?) niet met zekerheid waargenomen.

De enige andere Nederlandse soort met zo'n grove habitus als *E. borealis* is *E. suecica* Murb. & Wettst. *E. borealis* onderscheidt zich hiervan door de donkere bladen, de wigvormige voet der schutbladen (*E. suecica* met afgeronde voet), de smal driehoekige tanden van de vruchtkelk (*E. s.* met breed driehoekige tanden) en de langwerpige doosvrucht (*E. s.* breed elliptisch).

1. A. W. Kloos, De Nederlandsche Euphrasia's. Ned. Kruidk. Arch. 1920 (1921), p. 170–207.
2. H. W. Pugsley, A revision of the British Euphrasiae. Journ. Linn. Soc. London, 48, 1930, p. 467–544.
3. F. Townsend, Monograph of the British species of Euphrasia. Journ. of Bot. 35, 1897, p. 321–336, 395–406, 417–426, 465–477.
4. R. von Wettstein, Monographie der Gattung Euphrasia, 1896.

53. **Verbascum nigrum** L. var. **gymnostemon** Rchb.

Ottersum, Limb., in de buurtschap Zelder; van daar overgebracht naar een tuin te Soest, leg. *R. Tolman*, 10 sept. 1956 (L).

Deze variëteit is gekenmerkt door het ontbreken van de wollige beharing op de helmdraden. De opmerking van Hegi (1, p. 12), dat zij „wohl nur eine Folge von Insektenfrass" is, is zeker onjuist; men kan immers moeilijk aannemen, dat er insecten zijn, die zorgvuldig alle meeldraden van de vele bloemen van een plant geheel kaal scheren.

1. G. Hegi, Illustrierte Flora von Mittel-Europa VI, 1, 1913–18 (p. 5–112, 1913).

54. **Anthemis cotula** L. f. **discoidea** Baguet

Nieuw-Beerta, leg. *H. C. van Hall*, 10 aug. 1843 (NBV); Meers, gem. Elsloo, Limb., aan de Maasoever, leg. *Unio*, 21 juli 1955, herb. *v. O.* no. 18503 (L).

Een vorm van *A. cotula* L. zonder lintbloemen, die nog niet eerder uit ons land was vermeld, hoewel wij in het herbarium van de K.N.B.V. een exemplaar aantroffen, dat reeds in 1843 werd verzameld.

55. **Tragopogon dubius** Scop.

Reusel, N.Br., een vrij groot aantal exx. aan de wegrand bij het grenskantoor, leg. *J. H. Kern* no. 14374, 8 aug. 1955 (L); Nieuwe Sluis, gem. Groede, Zeel., een 40-tal exx. in de duinen ten W. van het afwateringskanaaltje, leg. *A. de Visser*, 9 juli 1957 (L).

De soort heeft onder het hoofdje een sterk verdikte, holle stengel en komt hierdoor overeen met *T. porrifolius* L.; zij verschilt echter van deze o.a. door de gele (niet violette) bloemen. *T. pratensis* L., eveneens met gele bloemen, heeft de stengel onder het hoofdje niet of nauwelijks verdikt. Verder zijn de vruchten met inbegrip van de snavel bij *T. dubius* 2½–4 cm lang en met duidelijkere en scherpere tandjes bezet dan de tot 2½ cm lange vruchten van *T. pratensis*.

De in de West-Europese literatuur onderscheiden ondersoorten, subsp. *dubius* en subsp. *major* (Jacq.) Vollm. zijn volgens MONTGOMERY (1) zonder enige systematische waarde; wij zijn het met deze opvatting eens.

T. dubius komt voor in Zuid- en Midden-Europa, westelijk en noordelijk tot in Noord-Frankrijk en Midden-Duitsland, en voorts oostelijk tot in Klein-Azië en de Kaukasus. Voorts werd zij volgens schriftelijke mededeling van Ir. J. E. de Langhe (Berchem, Antw.) ook reeds in België gevonden, o.a. bij Zeebrugge, 1939; St. Katelijne-Waver, 1954 en Nismes, 1955.

1. F. H. MONTGOMERY, A nomenclatural note in the genus Tragopogon. Rhodora 55, 1953, p. 325–328.

56. **Verbesina encelioides** (Cav.) Benth. & Hook. var. **encelioides.** (Fig. 4, *c*).

Tussen Wartena en Eernewoude, vuilstortterrein van de gemeentereiniging Leeuwarden, leg. *D. Franke & D. T. E. van der Ploeg*, 29 okt. 1955 (herb. *v. d. Ploeg;* L).

Van *Verbesina encelioides* was tot nu toe alleen de var. *exauriculata* Robins. & Greenm. (Fig. 4, *d*) bij ons adventief aangetroffen (Nijmegen, 1938, 1939; Grave, 1952, 1953). De nu gevonden var. *encelioides* verschilt hiervan door het bezit van oortjes aan de voet van de bladsteel. Het is waarschijnlijk, dat de plant werd aangevoerd met afval van een meelfabriek te Leeuwarden.

V. encelioides komt voor in de Verenigde Staten; volgens FERNALD (1, p. 1495) van Montana tot Arizona, oostelijk tot Kansas en Texas; adventief van Missouri tot New England. De var. *encelioides* is volgens hem de meer zuidelijke variëteit.

1. M. L. FERNALD in Gray's Manual of Botany, 8th ed., 1950.

57. **Potamogeton × decipiens** Nolte ex Koch (door E. W. CLASON). (Fig. 5).

Leekster Meer, Z.O.-hoek, één pol in open water ter diepte van ca. 1.50 m, leg. *E. W. Clason*, 7 juli 1955.

De pol van *P. × decipiens* viel op, doordat de spruiten minder vertakt waren dan die van *P. lucens* L. en *P. perfoliatus* L. in naburige pollen. De plant lijkt het meest op een kleinbladige *P. lucens* L., maar wijkt daarvan af:

1. door de afwezigheid van elke aanduiding van een bladsteel; de langwerpige bladschijf zit ter weerszijden van de middennerf met smalle stroken aan de stengel vast;

2. doordat de bladtop niet steeds een uitstekend puntje heeft, maar ook afgerond voorkomt; vooral bij de top vertoont de bladrand bij sterke vergroting hyaline uitsteekseltjes van de celwanden;

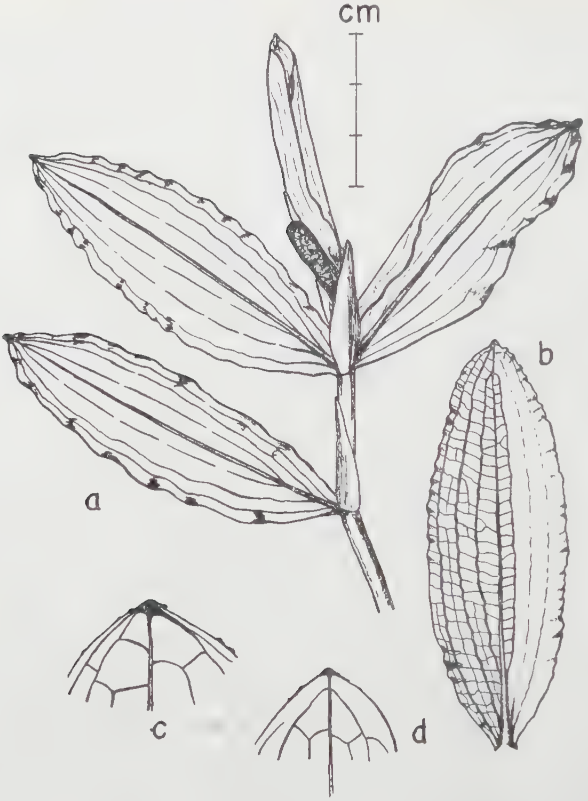


Fig. 5. *Potamogeton* × *decipiens* Nolte ex Koch, *a:* bloeistengel, *b:* bladnervatuur; *c–d:* bladtoppen, vergroot.

3. door de afwezigheid van kielen op de rug van het tongetje; de top van het tongetje is tamelijk lang uitgetrokken.

De beschrijvingen van Koch (7), Fryer (2, 3), Graebner (4, 5, 6) en Clapham (1) typeren *P.* × *decipiens* zodanig, dat het voor ons geen twijfel laat, dat we hier met dit voor Nederland nieuwe taxon te doen hebben. De naam is afkomstig van E. F. Nolte, Kiel, maar werd gepubliceerd door W. D. J. Koch (7, p. 779). Planten van dit taxon werden in Europa gevonden op de Britse Eilanden en van de Pyreneeën tot in Zweden, Lithauen en Tirol, en voorts vermeld voor delen van Azië.

Goed ontwikkelde vruchten zijn nimmer aangetroffen; onze plant had op 7 juli geen goed volgroeide bloeiaren, terwijl dat bij de naburige *P. lucens* en *P. perfoliatus* wel het geval was. Fryer (2, p. 137) [zie ook Fryer & Bennett (3, p. 59, 75, pl. 48)] heeft, voorzover ons bekend, het eerst geschreven over de bastaardnatuur van het taxon, dat hij zich voorstelt als een aggregaat van onafhankelijk van elkaar ontstane hybriden, die zich vegetatief handhaven.

Algemeen wordt aangenomen, dat *P. lucens* een der ouders is. Wat de andere ouder betreft zijn de inzichten verdeeld over *P. perfoliatus* L. en *P. praelongus* Wulf. Naar de kenmerken van onze plant te oordelen, is er veel te zeggen voor afstamming van *P. praelongus*, vooral vanwege de bladvoet. Bij de bladen met afgeronde top krijgt men soms de indruk, dat er iets te zien valt van de kapvormig samengetrokken bladtop van *P. praelongus*. Bovendien komt *P. praelongus* wel iets meer met *P. lucens* overeen dan *P. perfoliatus*, zodat een bastaard van *P. lucens* en *P. praelongus* dichter bij *P. lucens* zou staan dan de combinatie *P. lucens*-*P. perfoliatus*. Voor de afstamming van *P. perfoliatus* pleit in ons geval feitelijk alleen, dat *P. lucens* en *P. perfoliatus* op de vindplaats aanwezig zijn, hoewel *P. praelongus* in Leutingewolde vlak bij het Leekster Meer gesignaleerd werd (excursie Ned. Bot. Ver., 24 en 25 juli 1923; I.V.O.N.-archief). *P.* × *decipiens* heeft geen enkel specifiek *perfoliatus*-kenmerk; men zou bij een bastaard van deze soort toch iets verwachten van de stengelomvattende, hartvormige bladvoet, van de eironde in plaats van langwerpige bladvorm, van de vele zijdelingse lengtenerven en van het tere, kleine, spoedig verdwijnende tongetje, zoals *P. perfoliatus* zo kenmerkend vertoont. Fryer (2) concludeert tot de afstamming *P. lucens* × *perfoliatus* vooral op grond van het samen voorkomen met de veronderstelde ouders; Clapham (1, p. 1196) is nog van dezelfde mening; hij vermeldt: "Ponds, canals, rivers, etc., with the parents". Graebner (4, p. 329) nam oorspronkelijk de afstamming *P. lucens* × *perfoliatus* aan. Later brengt hij (5, p. 137) „*P. decipiens* Nolte in Koch, 1844 sens. str." onder *P. lucens* × *praelongus* en dit is sindsdien zijn standpunt gebleven (zie 6, p. 505).

Voor het probleem van het ontstaan van *P.* × *decipiens* is het van belang op te merken, dat er nog vier nauw verwante taxa zijn beschreven, die eveneens tot *P. lucens* × *praelongus* gebracht worden, en nog andere tot *P. lucens* × *perfoliatus* of *P. praelongus* × *perfoliatus*. Dit wijst wel op een ingewikkelde bastaardvorming, waarbij men ook fertiele taxa, zoals *P. praelongus* Wulf., en *P. zizii* Roth, als door bastaardering ontstaan kan beschouwen. Men moet ook bedenken, dat alle genoemde taxa voor zover bekend tetraploiden zijn (zie Clapham, l.c.) en derhalve ook afstamming van diploiden niet uitgesloten is.

1. A. R. Clapham, Potamogetonaceae, in A. R. Clapham, T. G. Tutin & E. F. Warburg, Flora of the British Isles, 1952, p. 1188–1209.
2. A. Fryer, Notes on Pondweeds. Journ. of Bot. 28, 1890, p. 137–139.
3. A. Fryer & A. Bennett, The Potamogetons of the British Isles, 1915.
4. P. Graebner in Ascherson & Graebner, Synopsis der Mitteleuropäischen Flora 1, 1897.
5. P. Graebner, Potamogetonaceae. Das Pflanzenreich IV, 11, 1907.
6. P. Graebner in Ascherson & Graebner, l.c., ed. 2, 1, 1913.
7. W. D. J. Koch, Synopsis Florae Germanicae et Helveticae, ed. 2, 2, 1844.

58. **Verwilderde cultuurplanten.**

a. **Helxine soleirolii** Req.

Woerden, in een bloemisterij, leg. *A. Eisses*, juli 1955 (L).

Deze wel als rotsplantje gekweekte *Urticacea* groeide volgens de vinder in grote hoeveelheid in een bloemisterij te Woerden en was daar tot een vrijwel onuitroeibaar onkruid geworden.

De soort is afkomstig van Corsica, Sardinië, Caprera en de Balearen, waar zij gevonden wordt in beschaduwde, vochtige rotsspleten.

b. **Mahonia aquifolium** (Pursh) Nutt.

Wassenaar, Voorlinden, in de duinen, leg. *S. J. van Ooststroom* no. 19849, 29 sept. 1956 (L); 's-Gravenhage, Westduinen, leg. *R. M. van Urk*, 3 okt. 1956 (L).

Deze veel gekweekte sierheester kwam op de genoemde plaatsen verwilderd voor. Besetende vogels zullen wel een rol gespeeld hebben bij de verspreiding.

De soort komt oorspronkelijk voor in westelijk Noord-Amerika, van Brits Columbia tot Oregon en Idaho.

c. **Barbarea vulgaris** R.Br. var. **plena** Wildsmith

Tussen Obbicht en Grevenbicht, aan de Maasoever bij de grintgroeve, leg. *I.V.O.N.-exc.*, 7 juni 1955, herb. *v. O.* no. 18205 (L).

Deze variëteit met gevulde bloemen wordt volgens Boom (1, p. 118) soms als sierplant gekweekt. De weinig of niet fertiele bloemen behouden lang hun kroonbladen en vormen tenslotte fraaie lange, gele trossen.

1. B. K. Boom, Flora der Cultuurgewassen van Nederland 2, 1950.

d. **Tolmiea menziesii** (Pursh) Torr. & Gray

IJsbrechtum, Fr., bos Epemastate, leg. *Huese*, 20 juli 1955 (herb. *van der Ploeg*); id., leg. *D. A. Tamminga*, 15 juni 1957 (L); in een bos bij Ede, Gld., leg. *Mevr. A. A. Zwart–de Graaf*, mei 1957 (L).

Deze gewoonlijk als kamerplant gekweekte, onder de naam van „Kindje op moeders schoot" bekende, *Saxifragacea* is op beide bovengenoemde plaatsen in een bos verwilderd en lijkt zich, althans te IJsbrechtum, goed te handhaven.

Tolmiea menziesii is een bosplant uit westelijk Noord-Amerika, waar zij voorkomt van Alaska tot Californië.

e. **Cytisus supinus** L., s.lat.

Vasse, Ov., in groot aantal op een heideveld, leg. *P. Jansen & W. H. Wachter*, aug. 1923 (L).

Een tweetal takjes, behorende tot deze soort, zoals zij door Briquet (1, p. 173) en later door Ascherson & Graebner (2, p. 327) wordt omgrensd, troffen wij aan in het zich in het Rijksherbarium bevindende herbarium Jansen & Wachter onder de onjuiste naam *Cytisus monspessulanus* L. Aan het materiaal is niet met zekerheid uit te maken tot welke ondersoort van *C. supinus* het behoort. Aan deze vondst is blijkbaar nooit ruchtbaarheid gegeven. Het zou de moeite waard zijn om na te gaan of de soort, die zoals het etiket aangeeft, in groot aantal voorkwam, nadien nog is teruggevonden of misschien heeft standgehouden.

De soort (s.lat.) is inheems in Spanje, Frankrijk, Zuid-Zwitserland, Noord-Italië, het Balkan-schiereiland, Klein-Azië en Syrië.

1. J. Briquet, Études sur les Cytises des Alpes Maritimes, in E. Burnat, Mat. pour serv. à l'hist. fl. Alp. Mar., 1894.
2. P. Ascherson & P. Graebner, Synopsis der Mitteleuropäischen Flora 6, 2. Abth., 1907.

f. **Oxalis deppei** Lodd.

Maarn, in spoorweguitgraving, leg. *S. J. van Ooststroom* no. 19826, 28 aug. 1956 (L).

O. deppei, het zg. geluksklavertje, een *Oxalis* met 4-tallige bladen en rode bloemen, werd op genoemde vindplaats op een ruderale plek aangetroffen, samen met andere verwilderde sierplanten.

De soort is afkomstig uit Mexico.

g. **Phacelia campanularia** A. Gray

Tussen Gameren en Kerkwijk (Bommelerwaard), op een akker met voor bijenvoer gekweekte *Phacelia tanacetifolia* Benth., op vrij zware kleigrond, een 10-tal exemplaren, leg. *H. Mooi & A. Westera*, 5 juli 1956 (L).

Deze uit Zuid-Californië afkomstige soort vertoont veel overeenkomst met de eveneens wel als eenjarige zaaibloem gekweekte en soms verwilderde *Phacelia minor* (Harvey) Thell. (= *Whitlavia grandiflora* Harvey). Zij verschilt hiervan o.a. door de onderaan niet verwijde bloemkroon, door de kleinere, kale schubben aan de voet van de helmdraden en door het kleinere aantal zaadknoppen (ca. 20–30) per placenta. Bij *P. minor* is de kroonbuis aan de voet verwijd, zijn de schubben groter en behaard en komen ca. 40–50 zaadknoppen per placenta voor.

h. **Cynoglossum amabile** Stapf & Drummond

Haarlem, op aardappelland aan de Frederik Hendriklaan, leg. *J. Brand*, 6 juli 1942 (L).

Deze zelden als sierplant gekweekte soort is afkomstig uit Tibet en West-China.

i. **Sagittaria graminea** Michx.

Dorst, N.Br., in een sloot waardoor koelwater van het pompstation van de waterleiding van Breda stroomt, leg. *P. Zonderwijk*, 26 okt. 1955 (L).

S. graminea wordt wel in warmwater-aquaria gekweekt en het is waarschijnlijk, dat de planten, die bij Dorst in een flink aantal op de genoemde vindplaats groeiden uit zo'n aquarium afkomstig zijn, temeer daar zij samen voorkwamen met een nog niet nader geïdentificeerde *Limnophila*-soort, die volgens VAN DRUTEN (1) zeker uit een aquarium afkomstig is. De vinder geeft op, dat de temperatuur van het slootwater het gehele jaar door belangrijk hoger is dan normaal. Het is duidelijk, dat zowel de *Sagittaria* als de *Limnophila* door de bijzondere omstandigheden ter plaatse een voor hun ontwikkeling geschikt milieu hebben gevonden en daardoor kans zien zich te handhaven.

1. J. F. VAN DRUTEN, Is de Ambulia bij ons inheems? Het Aquarium 22, 1951, p. 68.

SUMMARY.

The third series of "Floristische Notities" contains, with a few exceptions, additions to the flora of the Netherlands from the years 1955 and 1956. No. 52 of this series is written by Dr. A. G. de Wilde, Sassenheim, no. 57 by Mr. E. W. Clason, Groningen.

The series contains the following notes:

35, 44, 45, 46, 51, and 56. *Amaranthus polygonoides* L., *Psoralea americana* L., *Trifolium cernuum* Brot., *Trifolium rubens* L., *Gilia multicaulis* Benth. var. *peduncularis* (Eastw.) Jepson, and *Verbesina encelioides* (Cav.) Benth. & Hook. var. *encelioides*. First records of these aliens for the Netherlands, found in various localities; the numbers 35 and 45 near wool factories at Tilburg.

36. A specimen of *Caltha palustris* L. with flowers of the size of those of *Ranunculus acris* L. could possibly belong to var. *ranunculiflora* Schur.

37, 38, 39, 40, 41, 42, 43, 47, and 49. *Didesmus bipinnatus* (Desf.) DC., *Diplotaxis erucoides* (L.) DC., *Iberis parviflora* Munby, *Lepidium draba* L. subsp. *chalepense* (L.) Thell. var. *auriculatum* (Boiss.) Thell., *Matthiola* cf. *oxyceras* DC., *Sisymbrium septulatum* DC., *Hippocrepis unisiliquosa* L., *Trigonella caelesyriaca* Boiss., and *Pimpinella puberula* (DC.) Boiss. First records of these aliens for the Netherlands; all found along the river Meuse in the province of Limburg, near Obbicht, Grevenbicht and Meers, together with a large number of other adventitious species. It is not unlikely that diaspores of them at least partly have come from the wool factories along the Vesdre in Belgium.

48. Specimens of *Oenanthe aquatica* (L.) Poir. with purple flowers from the coast of the former Zuyderzee (IJselmeer). Possibly an autumnal form.

50. *Cuscuta australis* R.Br. found for the first time in the Netherlands, on *Polygonum bungeanum* Turcz. Earlier records of the species for this country appeared to refer to *Cuscuta campestris* Yuncker, as VAN OOSTSTROOM pointed out in Ned. Kruidk. Arch. 52, 1942, p. 165. The present authors do not agree with HJELMQVIST's view that the greater part of the Netherlands specimens of *C. campestris* should belong to *C. australis* R.Br. var. *cesatiana* (Bert.) Yuncker. They are of the opinion that the Swedish findings, mentioned by Hjelmqvist as belonging to this variety, fall within the variability of *C. campestris*, as do the plants that according to Hjelmqvist have been introduced in Sweden from Holland. Differential characters are given between *C. australis* and *C. campestris*.

52. *Euphrasia borealis* Townsend (by A. G. de Wilde). First record for the Netherlands. Discussion of differences between this species and *E. brevipila* Burnat & Gremli, *E. stricta* Host and *E. suecica* Murb. & Wettst.

53 and 54. *Verbascum nigrum* L. var. *gymnostemon* Rchb. and *Anthemis cotula* L. f. *discoidea* Baguet. First records for the Netherlands.

55. *Tragopogon dubius* Scop., a native of South and Central Europe was twice found in the southern part of the Netherlands. It seems not unlikely that this species will soon become naturalized with us.

57. *Potamogeton* × *decipiens* Nolte ex Koch (by E. W. Clason). First record for the Netherlands of this hybrid the putative parents of which are *P. lucens* L. and *P. praelongus* Wulf.

58. A number of plants escaped from cultivation.

Acta Botanica Neerlandica **7** (1958) 53—58

NOTES ON MYRTACEAE VII

MYRTACEAE OF FRENCH EQUATORIAL AFRICA

G. J. H. AMSHOFF

(*Laboratory for Plant Taxonomy and Plant Geography, Wageningen*)

(*received October 25th, 1957*)

While studying the *Myrtaceae* of Africa in the Paris herbarium, I found a few apparently new species among those collected in Gaboon and the French Congo. These regions are not covered by the Flora of West Tropical Africa, and none of the species of *Eugenia* known from West Tropical Africa north of the French Cameroons have so far been discovered in Gaboon or the French Congo. The descriptions of the new species follow here:

Eugenia Tholloni Amsh. n.sp. Fig. 1.

Frutex ramosus ramulis juvenilibus brunneo-tomentosis, adultis glabris, rimosis; internodiis brevibus circ. ½–1½ cm longis. Foliorum petioli brevissimi, circ. 2 mm longi, pubescentes, glabrescentes. Lamina oblanceolata, apice obtusa, basi attenuata, tenuiter coriacea, novella utrinque praesertim ad costam pubescentia, adulta glabra, tenuiter coriacea, 3½–6 cm longa, ½–1½ cm lata, costa supra plana, subtus prominente, nervis lateralibus tenuibus, utrinque circ. 8, patentibus, paullulum obliquis, in nervum collectivum circ. 1 mm a margine distantem conjunctis. Inflorescentia glomerata, floribus sessilibus vel interdum nonnullis breviter pedicellatis. Sepala rotundata, sparse pubescentia vel glabra, 1½ mm longa, ciliata. Stylus filiformis; stigma parvum, saepe breviter bifidum. Bacca globosa, in sicco ± 6 mm in diametro. Embryo homogeneus, rectus, nudus.

GABOON: Ogooue R., between the rocks of the rapids, Thollon 767 (P); 807 (P); 808 fl.fr., type (P).

A very distinct species, characterized by its oblanceolate, obtuse leaves and glomerate, pubescent flowers. The narrow leaves and the habitat suggest that this species is a typical rheophyte, i.e. a plant restricted to riverbeds with swiftly running water, and adapted to this habitat by the development of narrow leaves and a strongly branched rootsystem.

Eugenia librevillensis Amsh. n.sp.

Arbor 7–9 m alta. Ramuli novelli fere glabri; internodiis apice pàullulum dilatatis. Folia elliptica, basi acuta, apice abrupte acuminata, herbacea, glabra, 5–10 cm longa, 3–4(–4½) cm lata, costa supra impressa, subtus prominente, nervis lateralibus utrinque 9–10, tenuibus, patentibus, in nervum marginalem 2–3 mm a margine distantem conjunctis. Petiolus gracilis, ± 4 mm longus. Inflorescentia

breviter racemosa, dense florifera, rachide dense bracteata, circ. 5 mm longa, bracteis tomentosis, ± 1 mm longis. Pedicelli circ. 5 mm longi, griseo-tomentosi. Flores albi, hermaphroditi adhuc ignoti, ♂ cum vix ullo stylo. Ovarium basi tomentosum, in flore ♂ excavatum. Sepala rotundata, glandulosa, glabra. Petala circ. 4 mm longa. Bacca ignota.

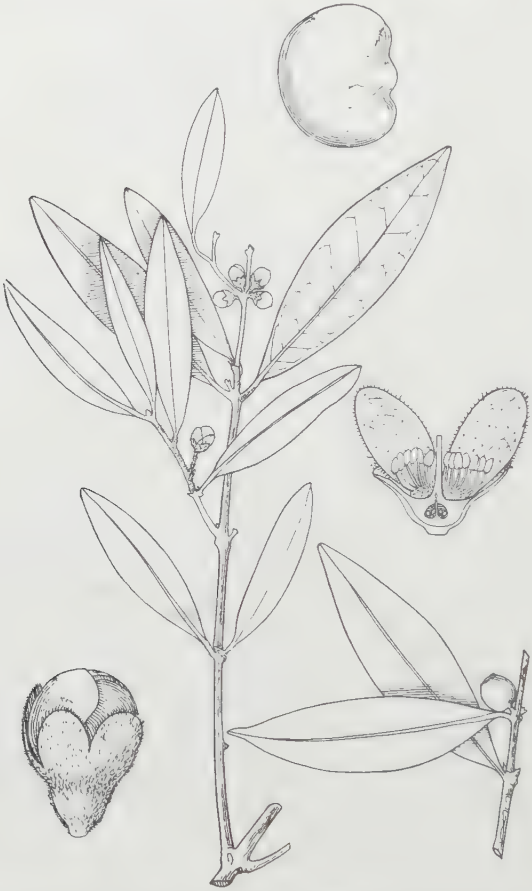


Fig. 1. *Eugenia Tholloni* Amsh. n.sp. Embryo, flowerbud and longitudinal section of the flower × 4.

Gaboon: near Libreville, Klaine 3509, type (P).

By its shortly racemose flowers, abruptly acuminate leaves, distinct pedicels and partly pubescent ovary this species seems to be sufficiently distinct. Its nearest ally is perhaps *E. Gilgii* Engl et v. Brehm., from the Cameroons, with obtuse or acutish leaves and quite glabrous ovary. The species may also be compared with *E. Kalbreyeri* Engl. et v. Brehm., which however has a more or less yellow indumentum

and the lateral nerves of the leaves more distinctly ascending and more strongly curved. Two other specimens collected by Klaine near Libreville, nrs. 1691 and 1692, may also belong to *E. librevillensis* Amsh., but have a scarcely developed rachis.

Eugenia diminutiflora Amshoff n.sp. Fig. 2.

Frutex glaber valde ramosus, ramulis dense foliatis, novellis breviter pubescentibus, internodiis brevibus circ. 1 cm longis. Folia breviter petiolata petiolo circ. 1 mm longo. Lamina elliptica, apice acuta vel inconspicue acuminata, basi acuta, glabra, tenuiter coriacea, 1 ½–3 cm longa, 7–15 mm lata, costa supra impressa, subtus prominente, nervis lateralibus utrinque circ. 5, obsoletis. Flores solitarii vel bini, glabri, pro genere minimi. Pedicelli circ. 1 mm longi, glabri. Ovarium vix 1 mm in diametro; sepalis minimis. Stylus filiformis; stigma acutum. Bacca ignota.

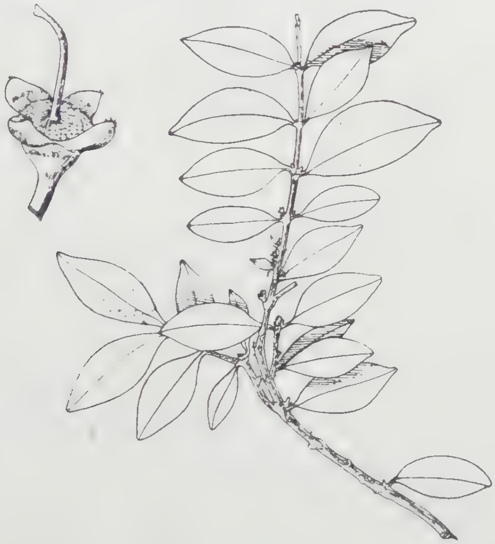


Fig. 2. *Eugenia diminutiflora* Amsh. n.sp. Deflorated flower × 12.

French Congo: without precise locality, Thollon s.n., type (P).

A very distinct species, well characterized by its densely foliate twigs, small leaves and minute flowers. *Eugenia rupestris* Engl. et v. Brehm., according to later authors merely a small-leaved, stunted form of *E. leonensis* Engl. et v. Brehm., has larger flowers and longer pedicels, the flowerbuds being described as 1 ½–2 mm in diameter and the pedicels as 3–4 mm long.

Eugenia gabonensis Amsh. n.sp.

Frutex vel arbor. Folia subsessilia, magna, 18–20 cm longa, circ. 6 cm lata, obovato-oblonga, basi subcordata vel rotundata, apice obtuse acuminata, glabra, costa crassa, nervis lateralibus utrinque circ. 12–15. Petiolus brevissimus, incrassatus. Flores ignoti. Bacca subsessilis, globosa, sepalis minimis coronata. Embryo rectus, homogeneus, sed fissura brevi instructus.

GABOON: near Libreville, Klaine 2827 fr. (K., P.), 2737 fr. (P); without further locality, Jolly 66 (P).

This species has sometimes been confused with *E. Klaineana* (Pierre) Engl., a still imperfectly known, but at any rate quite distinct species with distinctly petiolate leaves that are acute at their base. Both species agree in having an embryo with a short split at one side, which is an unusual character.

So far, seven species have been found in Gaboon and the French Congo, the three other species already described in earlier publications. A key to these species may run as follows:

1 a Leaves very small, not more than 3 cm long. Flowers minute, glabrous, their pedicels about 1 mm long . . . . . . *E. diminutiflora* Amsh.
b Other species 2
2 a Leaves 3–6 cm long, obtuse or somewhat obtusely acuminate 3
b Leaves 5–10 cm long, or even larger still, distinctly acuminate 4
3 a Flowers glomerate, brown-pubescent . . . *E. Tholloni* Amsh.
b Flowers borne on 3–10 mm long pedicels, glabrous . . . . *E. congolensis* De Wild. et Durand
4 a Leaves subsessile, subcordate or rounded at the base, 18–20 cm long . . . . . . *E. gabonensis* Amsh.
b Leaves distinctly petiolate, acute or obtuse at the base . . . 5
5 a Flowers in short racemes or fascicles, and borne by up to 5 mm long greyish-tomentose pedicels *E. librevillensis* Amsh.
b Flowers glomerate, subsessile or very shortly pedicelled; pedicels, if any, glabrous 6
6 a Young twigs pubescent. Leaves pubescent on the midrib beneath, at least when young. French and Belgian Congo . . . . . . *E. Demeusei* De Wild.
b Twigs scaly, glabrous even when young. Leaves quite glabrous. Gaboon. . . . . . . *E. Klaineana* (Pierre) Engl.

Eugenia Klaineana (Pierre) Engl., Pflanzenwelt Afrikas III.2 (1921) p. 735; — *Chloromyrtus Klaineana* Pierre in Bull. Soc. Linnéenne Paris n.s. I (1898) p. 71; — *Eugenia Soyauxii* Engler in Notizblatt II (1899) p. 291 p.p.; A. Chevalier, Bois du Gaboon (1911) p. 211.

GABOON: Environs de Libreville, Klaine 128 and 1224, type of *Chloromyrtus Klaineana* Pierre in Bull. Soc. Linnéenne Paris n.s. I (1898), p. 71; Minda region, near Sibanga farm, Soyaux 109, 162, named *E. Soyauxii* Engl. by Engler l.c. (P).

Chloromyrtus Klaineana Pierre was reduced by Niedenzu in E. P. Nachtr. II zu III 7 (1900) p. 49 to *E. Soyauxii* Engl.; the combination *Eugenia Klaineana* however was apparently used for the first time in 1917 by Engler in Engler, Bot. Jahrb. LIV (1917) p. 339 and in Engler, Pflanzenwelt Africas III.2 (1921) p. 735; Engler however omitted to cite either Pierre's name or his original description.

Englers description of *E. Soyauxii* Engl. is partly based on Soyaux 109 and 162a, which indeed belong to *E. Klaineana* (Pierre) Engl., and partly on Kalbreyer 157, afterwards made by Engler and v. Brehmer in Engler, Bot. Jahrb. ILV (1917) p. 291 the type of a distinct species, *E. Kalbreyeri* Engl. et v. Brehmer.

Eugenia Demeusei De Wild. in Ann. Mus. Congo Serie V.2 (1908) p. 325; Durand, Syll. Fl. Congo (1908) p. 202; — *Eugenia landolphoides* A. Chevalier, Etudes Fl. Afrique Centr. francaise I (1912) p. 127, nomen nudum; Engler, Die Vegetation Afrikas Bd. III.2 (1921), p. 734 in key.

This species can perhaps best be compared with *E. calophylloides*, the most common species of West Tropical Africa. In *E. calophylloides* however, the young leaves are beautifully brown-pubescent, the adult leaves on the lower surface evenly covered with short decolorated hairs but otherwise glabrous, the flowers pubescent. *Eugenia Demeusei* is known from the Belgian Congo also.

Eugenia congolensis De Wild. et Th. Durand in Th. Durand, Mat. Fl. Congo V (1899) p. 5.

French Congo: North of Stanley Pool, De Wevre 719 (BR), type of *E. congolensis* De Wild. et Durand.; Gaboon, Ogooue R., Thollon 771.

This species is known from the Belgian Congo and from Angola too; it is however still imperfectly known, and possibly not easily distinguisable from the Rhodesian *E. chirindensis* Baker.

Eugenia liberiana Amsh. nomen novum; — *Eugenia calycina* Bentham, Fl. Nigrit. (1849) p. 358, non *Eugenia calycina* Camb. 1829.

A worldwide satisfactory revision of *Eugenia* L. has not yet been accomplished, but it seems safe to say that an apparently undivided embryo, a few-flowered centripetal inflorescence and a calyx limb not produced above the ovary are essential characters of the genus. The American species of the genus *Eugenia* L., moreover, have the following characters in common (cf. Merrill and Perry in Journ. Arn. Arb. 19 (1938) p. 99–100).

Embryo apparently undivided, pseudomonocotyledonous; seed coat smooth and free from the pericarp; inflorescence centripetal with the pedicels 1-flowered; flowers hermaphrodite; petals free; stigma punctiform; calyx limb not produced above the ovary. If we characterize the genus *Eugenia* L. in this manner, the American genus *Anamomis* Griseb. (*E. fragrans* (Sw.) Willd. and allies) with few-flowered, centrifugal inflorescence and free cotyledons, must be accepted as distinct.

The African members of the genus *Eugenia* L. agree with the American species in having an apparently undivided embryo; this embryo however is naked, with the seedcoat probably adhering to the pericarp, as in *Syzygium* Gaertn. It is however, not certain that all American species of *Eugenia* L. have a free testa. The inflorescence of the African species is quite typical, though the pedicels may be

3-flowered (e.g. in *E. albanensis* Sond., in *E. Woodii* Dümmer, and in some specimens of *E. malangensis* (Hoffm.) Engl.).

As peculiarities of the African species can be noted that the stigma is often capitate or (*E. coronata*) orbicular or (*E. Aschersoniana* Hoffm. and *E. mossambicensis* Engl.) bifid, not always punctiform. The flowers are as a rule polygamous or (in *E. Tisserantii* Aubréville et Pellegrin according to the collector) dioecious. If the flowers are polygamous, there may be several short-styled male flowers and one or two long-styled hermaphrodite flowers in one fascicle. In *E. calophylloides* DC, a quite common West African species, the flowers are apparently always shortstyled; therefore these flowers are apparently at least partly fertile. For *E. albanensis* Sond. it is stated that the style is in the beginning very short, but lengthens afterwards; these flowers therefore are apparently protandric. Observations in the field are needed to throw light on these questions, and on the mode of pollinisation.

In Africa, there is no reason at all to unite *Syzygium* Gaertn. with *Eugenia* L., all African species of the genus *Syzygium* can be easily distinguished by their free cotyledons and ± manyflowered, centrifugal inflorescence and cohaerent petals. The genus *Jambosa* DC. appears to be restricted to Asia, with no representatives either in Africa or in America; although it has free petals and a distinct plumule, it is usually united with *Syzygium* Gaertn. (Miss Perry).

Most African species of *Eugenia* are shrubs or small trees, but especially interesting, as already indicated by Engler, Pflanzenwelt Afrikas IX Bd. 3.2 (1922) p. 731, are the geophytic species, which develop annual herbaceous shoots from woody subterranean rootstocks. To this group belongs a.o. *E. malangensis* (Hoffm.) Engl. (including, in my opinion, *E. Laurentii* Engl. and *E. angolensis* Engl.) from the Belgian Congo, Angola, Rhodesia, Nyassaland and Tanganyika, but not yet found in French territory.

Acta Botanica Neerlandica **7** (1958) 59–60

BEMERKUNGEN ZU DER MITTEILUNG VON Th. J. STOMPS

OENOTHERA BIENNIS VAR. HEMIKLEISTOGAMA

O. RENNER
(München)

(eingegangen am 16. Januar, 1958)

Herr Professor Stomps hat in dieser Zeitschrift (vol. 6, S. 378–380) über die Auffindung einer angeblichen neuen Mutante von *Oenothera biennis* berichtet und meine Auffassung von der Beziehung zwischen *Oe.biennis* und *Oe.hungarica* Borb. (= *Bauri* Boedijn) kritisiert.

Seine Angaben über die neue Form sind folgendermaßen in die Sprache der Oenotheren-Genetik zu übersetzen. Die Kreuzung *biennis* × *hemikleistogama* hat glatte Blätter wie der Vater, also enthält der Pollen nicht *rubens*. Die Kreuzung *hemikleistogama* × *biennis* wird so hoch wie die Mutter, also enthalten die Eizellen nicht *albicans*. Gleichzeitige Mutation in beiden Komplexen der *Oe. biennis* ist außerhalb aller Wahrscheinlichkeit, ein Bastard der *Oe. biennis* kann nach dem züchterischen Verhalten auch nicht vorliegen, also handelt es sich um eine Form, die nicht von *Oe. biennis* abstammt. Ob die Sippe homozygotisch oder, was wahrscheinlicher, komplexheterozygotisch ist, hätte sich durch die Ermittlung der meiotischen Konfiguration in wenigen Stunden entscheiden lassen. Ein Analogon des solitären Auftretens ist das einzige Exemplar der *Oe. purpurata*, das Klebahn unter *Oe. biennis* fand; bis heute weiß niemand, woher der eine Same kam und wo die eigenartige homozygotische Art zuhause ist.

Der Charakter hemikleistogam ist in beiden reziproken Kreuzungen mit *Oe. biennis* rezessiv, das Gen muß also in der neuen Sippe homozygot vorhanden sein. Daß die Bastarde bei Selbstung keine hemikleistogamen Individuen abspalten, beweist, daß das rezessive Gen an die tragenden Komplexe gebunden bleibt; beide Bastarde hätten mit der "Varietät" rückgekreuzt werden sollen. Zu einer oberflächlichen Analyse einer neuen *Oenothera*-Form ist die Verbindung mit mindestens einem halben Dutzend der gut bekannten Arten nötig.

Die neue "Varietät" soll in der Tracht der *Oe. hungarica* ähneln, die zudem auch "hemikleistogam" ist. *Oe. hungarica* gehört zu der Sammelart *Oe. strigosa* von Cleland und Munz, die von den zahlreichen Typen der Sammelart *Oe. biennis* sehr verschieden ist. Stomps meint, *Oe. hungarica* könne aus *Oe. biennis* hervorgegangen sein, wie Boedijn (1924!) ohne jeden Versuch der Analyse geäußert hat. Nach der Meinung von Stomps wären sogar zwei Mutationsschritte — in der Sprache der Oenotheren-Genetik zwei Segmentverwechslungen oder reziproke Translokationen —, eine Mutation an *albicans* und eine an *rubens*, imstande, von *Oe. biennis* zu *Oe. hungarica* zu führen. Ein

Blick auf die Chromosomenformeln der beteiligten Komplexe zeigt, daß das unmöglich ist.

albicans	11.13	1.4	2.14	3.6	5.7	9.8	10.12
laxans	11.13	1.2	3.10	4.7	5.8	6.9	12.14
rubens	8.14	1.2	3.11	4.9	5.6	7.12	10.13
undans	8.14	1.4	2.3	5.10	6.7	9.13	11.12

Nachschrift

Ich spreche der Redaktion meinen Dank aus für die Aufforderung, hier noch ein kurzes Wort folgen zu lassen; ich möchte nur Folgendes bemerken. Den Namen *O. Bauri* Boed., der mir auch mehr zusagt, habe ich beibehalten, weil *O. hungarica* Borb. nur ungenügend beschrieben worden ist. Es ist m.E. doch wohl sehr unwahrscheinlich, dass es sich nicht um eine Mutation handelt, wenn jemand Jahre lang Hunderte von Exemplaren von *O. biennis* in seinem Garten gezüchtet hat und plötzlich ein abweichendes Individuum auftritt. Auffallend ist auch die Ähnlichkeit zwischen den Diagnosen dieser neuen Form und der *O. Bauri*. Ihre Zytologie habe ich allerdings noch nicht studiert, aber dazu wird es hoffentlich noch kommen.

Th. J. Stomps

Acta Botanica Neerlandica **7** (1958) 61–68

PHYSIOLOGISCHE UNTERSUCHUNGEN ZUR REIFETEILUNG

II. Mitteilung

ÜBER DIE ÄNDERUNG DES NUKLEINSÄURENGEHALTES WÄHREND DER POLLENMEIOSE UND POLLENENTWICKLUNG VON *LILIUM HENRYI*

H. F. LINSKENS
(*Botanisches Laboratorium der R.K. Universität Nijmegen-Nederland*)

(*eingegangen am 31. Januar 1958*)

1. Einleitung

Das Problem der Induktion der Meiose in einem sich mitotisch teilenden Gewebe ist ein Grundproblem biologischer Forschung überhaupt. Sind doch mit diesem cytologischen Prozess einerseits der Umbau der Chromosomen, die Umordnung der Genome und die Reduktion des Chromosomensatzes verbunden, andererseits der Vollzug der sexuellen Fortpflanzung (Oehlkers 1956) gekoppelt. Für die Kausalanalyse des Induktionsprozesses bieten die Antheren höherer Pflanzen ein besonders gutes Objekt, weil die Meiosis dort stets relativ leicht und sicher zu erkennen ist.

In unserer ersten Mitteilung (Linskens 1956) haben wir als Vorarbeit für eine Gewebekultur der Pollenmutterzellen einige physiologische Zustandsgrössen im Zusammenhang mit dem Ablauf der Pollenmeiose und der Pollenentwicklung untersucht. Daraus wurde klar, dass im Verlauf der genannten Prozesse beträchtliche Umsetzungen stattfinden, die für den geordneten Ablauf der Reifeteilung und die normale Entwicklung der Pollen wesentlich sind, jedoch keinen Zusammenhang mit der Induktion der Meiose erkennen lassen. Nachdem es Huskins und Mitarbeitern (1948a, b, c, 1949, 1950) durch Einwirkung von Natrium-Nukleinat gelungen war, "*meioseähnliche*" Stadien zu erzeugen, schien es aussichtsreich, die Änderungen im Nukleinsäuren-Gehalt in den verschiedenen Stadien der Reduktionsteilung zu analysieren.

Über den Umbau der Nukleinsäuren im Zusammenhang mit der Kernteilung, insbesondere der DNS, liegen zahlreiche Arbeiten vor (z.B. Swift 1950; Bryan 1951; Ogur–Erickson–Rosen–Sax–Holden 1951; Taylor 1953; Taylor–McMaster 1954; Grun 1956). Sie registrieren mit verschiedenartigen Methoden den RNS-bzw. DNS-Gehalt einzelner Zellen und Zellkerne. Es erschien uns jedoch bedeutsam, auch den *Desintegrationsprozess, den die Tapetumzellen während der Meiose und der Pollenreifung* durchmachen, gleichzeitig zu erfassen. Die Zellen des Archespors lösen sich ja schon, bevor die Meiose in ihnen abläuft, aus dem Gewebeverband los und sind dann von einer "Nährlösung" umgeben; diese wird zunächst von den Tapetumzellen sezerniert und später von deren Zerfallsprodukten gebildet. Über die chemische Zusammensetzung und physiologische Funktion liegen bislang nur mikroskopische Beobachtungen vor. Aus den Stoffen, die durch die Sekretion und den Zerfall der Tapetumzellen frei werden, müssen die Gonotokonten jedoch während ihrer Teilungsvorgänge ihr Material auf dem Wege der Diffusion entnehmen. Nach der Loslösung aus dem Gewebeverband befinden sich die Archesporzellen ja im freien Raum des Loculus, nur gesteuert von der genetischen Konstitution und den stofflichen Prinzipien der "Nährlösung". Hier ist also ein *kompliziertes Zusammenspiel* zu erwarten (OEHLKERS 1956). Fehlsekretionen des Tapetums infolge genetisch bedingter Störungen der ernährungs-physiologischen Vorgänge führen daher zu Sterilitätserscheinungen im männlichen Geschlecht (WELZEL 1954). Für den Ablauf der Teilungsvorgänge liegt dabei exakte Synchronisierung in einem Pollenfach vor, die auf einem *diffusiblen stofflichen Steuerungssystem des Tapetumbreies* beruhen muß.

Änderungen in der gesamten "Gonotokonten-Aufschwemmung" versprechen daher am ehesten Aufschluß über ein induzierendes Prinzip zu geben. Nachdem sich Nukleinsäuren-Verbindungen als für die Auslösung "meiose-ähnlicher" Stadien wirksam erwiesen, untersuchten wir im gesamten Antheren-Inhalt dieselben in Zuordnung zu den zytologischen Stadien der Meiose.

2. METHODEN

Es wurden die gleichen Pflanzen von *Lilium henryi*, wie zu der ersten Untersuchungsreihe, verwendet (LINSKENS 1956). Die Bestimmung der einzelnen Entwicklungs- und Meiose-Stadien erfolgte stets an Teilen von 2 Antheren je Knospe. Eine Korrelation zwischen den zytologischen Zuständen und der Knospen- bzw. Antheren-Länge, wie sie ERICKSON (1948) gefunden hatte, ließ sich an unserem Material nicht zeigen. Auch sind die Wachstumsbedingungen in den verschiedenen Jahren so unterschiedlich, daß eine sichere Zuordnung auf Grund von Längenmessungen nicht möglich ist (Abb. 1).

Nach der zytologischen Charakterisierung und Längenmessung wurden die Inhalte der Antheren auf Objektträger ausgepreßt und unmittelbar verarbeitet. Zur Blockierung der Nukleasen wird bei NS-Bestimmung stets sofort 0,02 m NaF zugesetzt (vgl. EBERHARDT 1952). Das Trockengewicht wurde durch Differenzwägung nach Trocknung von 4 h bei 105 Grad C und Abkühlen über Chlorkalzium ermittelt. Stickstoffbestimmung erfolgt als Mikromethode nach KIELDAHL–PREGL.

Die Nukleinsäuren (NS) wurden mit 0,5 m Perchlorsäure bei 75 Grad C in 30 min. extrahiert (Aufarbeitungsschema: vgl. KANDLER et al. 1956). Die quantitative Bestimmung der RNS erfolgte nach HAHN und v. EULER (1946) mit Phloroglucin und Eisessig unter Zusatz von Fe(III)Cl_3 zur Vertiefung der blaugrünen Färbung. Die Farbintensität wurde bei 587 mμ mit dem Zeiss-Spektralphotometer ermittelt und die RNS-Werte an Hand einer Eichkurve mit Hefenukleinsäure (Hoffmann-La Roche) gewonnen. Die Bestimmung der DNS erfolgte ebenfalls an Hand der Kohlenhydratkomponente im

Anschluß an die Hydrolyse in 5 % Trichloressigsäure (30 min kochendes Wasserbad) nach Dische (1930) und Webb-Levy (1955) mit 5 % iger TCE und 0,5 % p-Nitrophenylhydrazin in Äthanol. Nach erneutem Erhitzen auf dem Wasserbad (20 min), Abkühlen, Extraktion mit n-Butylacetat und Zugabe von 2 n NaOH wird die Färbung sofort bei 560 mμ bestimmt. Die DNS-Werte lassen sich einer Eichkurve mit Thymonukleinsäure (Th. Schuchhardt) entnehmen. Zur Nukleotid-Bestimmung wurden diese entweder mit 8 %iger Uranylacetat-Lösung gefällt oder die NS durch Fällung mit 83,5 % Ba $(ClO_4)_2$-Lösung in Wasser (Fiers–Stockx 1957), Äthanol oder 2 % iger wässriger NaCl-Lösung entfernt.

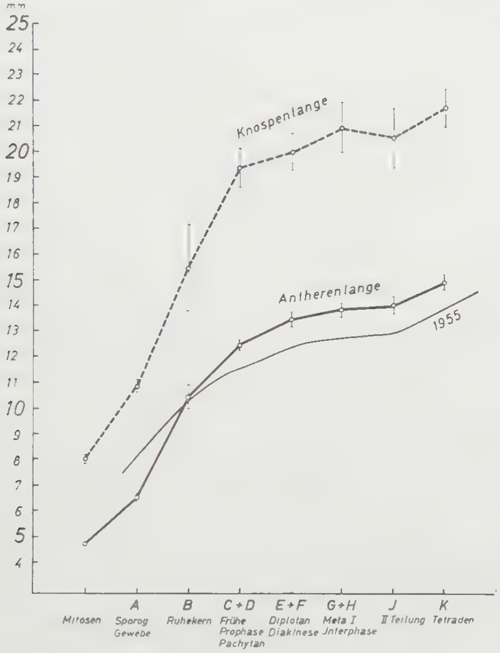


Abb. 1. Längenänderungen der Knospen und Antheren von *Lilium henryi* während der Pollenmeiose. Die Vergleichskurve für die Antherenlängenmessungen des Jahres 1955 (vgl. Linskens 1956) ist dünn ausgezogen.

Die papierchromatographische Trennung gelang mit verschiedenen Lösungsmitteln (Linskens 1955b); besonders geeignet waren: Phenol-Ameisensäure-Wasser (90:10:100), tertiäres Butanol-Salzsäure-Wasser (70:13,2:16,8), n-Butanol-Dioxan-Wasser (4:1:1; NH_3-Atmosphäre). Der Nachweis erfolgte mit Hilfe des Photoprintverfahrens. Die Vergleichssubstanzen und die Nukleinsäure-Baustein-Flecken ließen sich auch auf dem Papier direkt im UV-Licht (Spezialanalysenlampe Hanau) sichtbar machen durch Aufsprühen des Leuchtstoffes "S 5 grün" (Zn_2SiO_4 und MnO_4 in Subitogen) als Chloroform-Suspension (Wallenfels–Christian 1953, Linskens 1955a).

In den graphischen Darstellungen sind die 3fachen mittleren Fehler des Mittelwertes durch die Länge senkrechter Striche angegeben; fehlen diese, so liegt nur eine Einzelmessung vor.

Die Versuche wurden während der Vegetationsperioden 1956 und 1957 am Botanischen Institut der Universität Köln durchgeführt. Herrn Direktor Professor Dr. J. Straub gilt mein aufrichtiger Dank. Frau F. Tietz hat wiederum mit unermüdlichem Interesse die zytologischen Analysen gemacht, Fräulein I. Stahl hat 1956 mit großer Sorgfalt die chemischen Analysen ausgeführt; Herr Gartenmeister H. Schwamborn vermehrte und betreute die Versuchspflanzen; für diese Hilfeleistungen danke ich herzlich. Die Deutsche Forschungsgemeinschaft hat durch ihre Unterstützung die Fortführung der Untersuchungen im Rahmen des Schwerpunktprogrammes "Genetik" (vgl. DFG-Forschungsbericht Nr. *1*, 39, 1957) ermöglicht. Für diese Förderung sage ich meinen besten Dank.

3. Ergebnisse

Der *Wassergehalt* des Antheren-Inhaltes bleibt bis zur Tetradenbildung ziemlich gleich, sinkt aber dann stetig mit dem Eindicken des Tapetumsaftes ab (steigendes Trockengewicht, Abb. 2). Vergleicht man die Trockengewichtsänderung der Gesamtanthere (Linskens 1956, Abb. 2), so wird ersichtlich, daß die Auflösung und Verflüssigung des Tapetumgewebes nicht durch zusätzliche Wasseraufnahme, sondern durch Desintegration des Zellinhaltes des Tapetums zustande kommt.

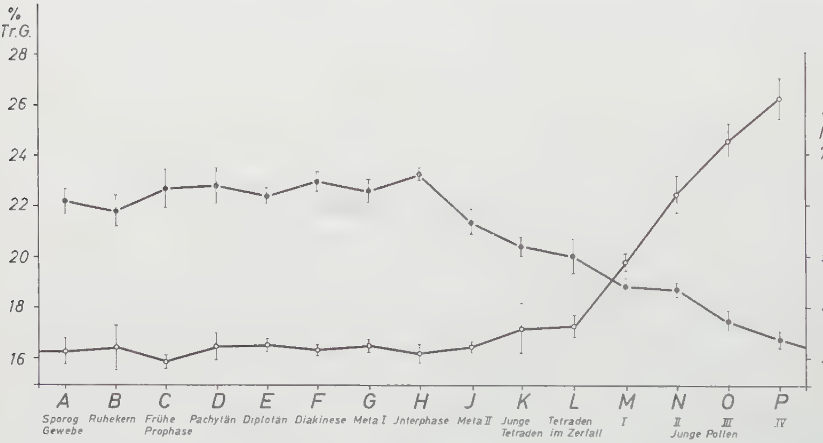


Abb. 2. Änderungen des Trockengewichtes und des Stickstoffgehaltes für den Antheren-Inhalt von *Lilium henryi* während der Pollenmeiose und Pollenentwicklung. Trockengewicht: —○— und linke Ordinate. N-Gehalt in % des Trockengewichtes: —●— und rechte Ordinate.

Der *Stickstoffgehalt* (Abb. 2) des Anthereninhaltes liegt mit rund 6 % im ersten Abschnitt der Meiose wesentlich über dem durchschnittlichen N-Gehalt der Gesamtanthere. Er bleibt bis zur Interphase auf dieser Höhe, um dann stetig abzufallen und erreicht im reifen Pollen rund 3 %.

Die Bestimmung der *Nukleinsäuren* (Abb. 3) erfolgte ebenfalls für den gesamtenAnthereninhalt. Dabei zeigte sich, daß im Stadium des sporogenen Gewebes (A) ein Gehalt an DNS, bezogen auf das Trockengewicht, von rund 1,1 % zu finden ist. Mit dem Einsetzen der Meiose in der frühen Prophase (Stadium C) sinkt jedoch der DNS-Gehalt, um sich vom Pachytän bis zur Metaphase (Stadien D bis G) auf rund 0,5 % des Trockengewichtes einzustellen. In der Interphase (Stadium H) erreicht der DNS-Gehalt wieder den Ausgangswert von rund 1,2 %, um mit der Tetradenbildung wieder abzufallen. In den jungen Pollen ist der NS-Gehalt ständig ansteigend. Der RNS-Gehalt des Antheren-Inhaltes ändert sich im zweiten Teil der Entwicklung (ab Interphase) synchron. Im ersten Abschnitt liegt jedoch der Anstieg der RNS zum Ausgangswert des ruhenden Gewebes bereits in der Diakinese.

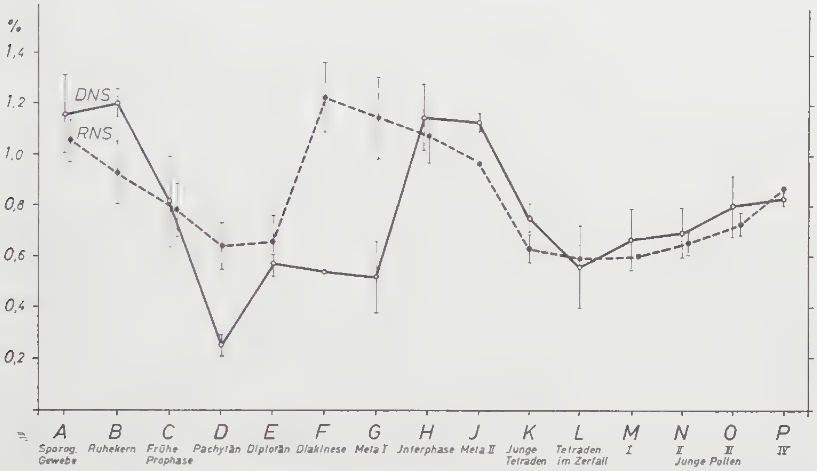


Abb. 3. Änderung des Gehaltes an Ribosenukleinsäure (RNS) und Desoxyribosenukleinsäure (DNS) im Anthereninhalt von *Lilium henryi* während der Pollenmeiose und Pollenentwicklung. Menge in % auf das Trockengewicht bezogen. ——●—— = RNS, ——○—— = DNS.

Die analysierten Mengen erfassten den Gehalt an NS der Pollenmutterzellen bzw. Tetraden bzw. Pollen *und* der Tapetum-Zellen. Der plötzliche Abfall mit dem Einsetzen der Reifeteilung läßt sich kaum mit den cytologischen Veränderungen *in* den Gonotokonten in Verbindung bringen. Es muß die schlagartige Verminderung der DNS speziell mit der Desintegration der Tapetum-Zellen und -Kerne in Zusammenhang stehen. Die Frage entsteht daher: wo bleiben die beim Zerfall des Tapetums frei werdenden Nukleinsäuren? Da sie als solche nicht gefunden werden konnten, liegt es nahe, nach ihren Bruchstücken zu fahnden. Wir trennten daher in einer weiteren Versuchsreihe durch Zentrifugieren aus der Aufschwemmung des Antheren-Inhaltes die Gonotokonten ab. Nach 2-maligem Waschen war im

Sediment das Material gut von anhaftenden Tapetumresten befreit. Mikroskopische Kontrolle der Tapetumzellen-Aufschwemmung zeigte in keinem Fall mehr das Vorhandensein von Gonotokonten bzw. jungen Pollen. Die resultierende Tapetum-Aufschwemmung wurde bei —40 Grad C durch Gefriertrocknung eingeengt und dann unmittelbar chromatographiert. Das Ergebnis ist in Tab. 1 wiedergegeben. Es ließen sich in dem Abschnitt der NS-Depression (Stadien D-G) eine Anzahl der verschiedensten Nukleotide und heterocyklischen Verbindungen im Tapetum bzw. seinen Degenerationsprodukten nachweisen und an Hand der vorhandenen Vergleichssubstanzen mit hoher Wahrscheinlichkeit identifizieren. In jedem Falle handelt es sich um Verbindungen, die im kurzwelligen UV (2540 Å) absorbieren, also als Nukleinsäurebruchstücke anzusehen sind. Die NS-Bruchstücke im Tapetumsaft treten zuerst in größerer Anzahl mit dem Pachytän (Stadium D) auf. Das zeitliche Zusammentreffen mit dem rapiden Absinken des NS-Gehaltes dürfte nicht zufälliger Natur sein. Als Interpretation scheint uns auf der Hand zu liegen, daß *die NS der Tapetumzellen, insbesondere die DNS, aus deren Kernen bei dem Desintegrationsprozeß unter dem Einfluß freigesetzter Nukleinasen und Nukleotidasen in ihre Nukleotide und kleinere Stücke aufgespalten werden.* Diese werden sodann von den in der Meiose befindlichen Gonotokonten aufgenommen und zu genuinen NS aufgebaut. Der Aufnahmeprozeß vollzieht sich während der Interphase und der II. Metaphase.

TABELLE I

Auftreten von Nukleinsäure-Bruchstücken während der Pollenmeiose und Pollenentwicklung von *Lilium henryi.* — = nicht beobachtet; + = stets beobachtet; ± = nicht regelmäßig gefunden. Die R_F-Werte der unbekannten Substanzen sind für das Verteilungsgemisch Phenol-Ameisensäure-Wasser angegeben.

	Adenylsäure	Guanylsäure	Cytidylsäure	Adenin	Guanin	Thymin	Unbekannte Substanzen		
							A $R_F =$ 0,01–0,2	B $R_F =$ 0,20–0,25	C $R_F =$ 0,25–0,40
A. Sporogenes Gewebe	—	—	—	—	—	—	+	—	—
B. Ruhekern	—	—	—	—	—	—	+	—	—
C. Frühe Prophase	—	—	—	±	±	—	+	—	—
D. Pachytän	+	+	+	+	+	+	+	+	+
E. Diplotän	+	+	+	±	+	+	+	+	+
F. Diakinese	+	+	+	±	+	+	+	+	+
G. Metaphase I	+	+	+	±	+	—	+	+	+
H. Interphase	—	—	+	—	+	—	+	+	—
I. Metaphase II	—	—	—	—	—	—	+	—	—
K. Junge Tetraden	—	—	—	—	—	—	+	—	—
L. Tetraden im Zerfall	—	—	—	—	—	—	+	—	—

4. Besprechung

Das Problem der Nukleinsäure-Synthese in der Meiose ist von hohem biologischen Interesse. An verschiedenen Objekten ist daher von zahlreichen Autoren eine Analyse mit Hilfe von autoradiographischen, histophotometrischen oder mikrochemischen Methoden durchgeführt worden. Eine kritische Besprechung haben PLAUT (1953) und TAYLOR (1957) vorgenommen; die auf verschiedene Weise gewonnenen Resultate stimmen nicht überein. Beim Vergleich der Analysenergebnisse ist zu berücksichtigen, daß bei Verfolgung der P^{32}-Aufnahme die *dynamische* Seite des Vorgangs unmittelbar erfaßt wird, während bei unseren Analysen in der Feststellung des prozentualen Gehaltes das *Ergebnis* des Syntheseprozesses zum Ausdruck kommt, also nicht zu entscheiden ist, ob es sich um Synthese oder Umsatz handelt. Unsere Ergebnisse klären weiterhin die Vermutungen von PLAUT (1953) und TAYLOR (1953) hinsichtlich der Inkorporation in eine instabile plasmatische Komponente. Darunter wäre etwa als erster Schritt der Einbau von Phosphor im Tapetummaterial zu verstehen. Es findet also offensichtlich ein *Übergang von Vorstufen aus den desintegrierenden Tapetumzellen auf dem Wege über eine Diffusion* statt. Für tierische Zellen besteht für diese Vorstellung infolge des Fehlens von Zellwänden kein Hindernis (KOLLER 1951). Es hat sich jedoch neuerdings zeigen lassen, daß der pflanzliche Kern durch die Kernmembran hindurch während der meiotischen Phasen durch Poren mit dem Milieu in Kontakt steht (HASSENKAMP 1957; DE 1957) und auch die Zellwände der PMZ durch Poren und plasmatische Stege Verbindungen nach Außen haben (HASSENKAMP u. LIESE 1957). Es ist daher keine Schwierigkeit die *Passage von Tapetummaterial durch Poren der Kernmembran* anzunehmen (ANDERSON u. BEAMS 1956). Damit würde sich auch der von COOPER (1952) im zytologischen Bild beobachtete Transfer von feulgenpositivem Material erklären als *ein Übergang von Nukleinsäurebausteinen aus dem Tapetum in die Mikrosporocyten, wo sie sodann zu spezifischen DNS-Matrizen aufgebaut werden.*

Für den geordneten Ablauf der Meiose dürfte jedoch die Anwesenheit bestimmter Nukleotide und Nukleoside im Zusammenhang mit der DNS-Synthese eine entscheidende Voraussetzung sein. Damit sind neue Möglichkeiten für die in-vitro-Kultur von sporogenem Gewebe und die Induktion der Meiose angedeutet.

Ob und inwieweit die DNS-Bruchstücke der Tapetumzellen noch Träger genetischer Informationen sein können, darüber wäre reizvoll zu spekulieren. Für einen experimentellen Ansatz sind jedoch andere Objekte besser geeignet.

ZUSAMMENFASSUNG

Die Untersuchung der Nukleinsäuren des Antheren-Inhaltes (Gonotokonten *und* Tapetum) ergab, daß mit dem Einsetzen der frühen Prophase I und dem Beginn des Zerfalls der Tapetum-Zellen eine Abnahme des DNS-Gehaltes von 1,1 % des Trockengewichtes auf ca. 0,5 % erfolgt. In der Interphase I steigt der DNS-Gehalt schnell wieder auf den Ausgangswert an. In der Zeit der Depression kann in dem Anthereninhalt das Auftreten von Nukleinsäure-Bausteinen papierchromatographisch nachgewiesen werden. Es wird angenommen, daß ein Transfer der DNS aus dem Tapetum in die Mikrosporocyten auf dem Wege über die DNS-Bausteine erfolgt.

SAMENVATTING

Het onderzoek van de nucleine-zuren van de antheren-inhoud (gonotokonten *en* tapetum) wees uit, dat met het begin van de vroege prophase en het begin van de afbraak der tapetum-cellen een vermindering van het DNZ-gehalte van 1,1 % van het drooggewicht tot ca 0,5 % plaats vindt. Tijdens de interphase neemt het DNZ-gehalte weer snel toe tot de uitgangswaarde. Gedurende de tijd dat het DNZ-gehalte laag is, kan in de antheren-inhoud, na papierchromatographische scheiding, het voorkomen van nucleine-zuur-componenten aangetoond worden. Aangenomen wordt, dat overbrenging van DNZ uit het tapetum in de microsporocyten via de DNZ-componenten plaats heeft.

SUMMARY

The examination of the nucleic acids in the contents of the anthers (microsporocytes *and* tapetum) has shown, that the onset of the prophase I and the beginning of the crumbling of the tapetum-cells a decrease in the amount of DNA takes place from 1,1 % of the dry weight to ca. 0,5 %. In the interphase I the amount of DNA returns to the original level. At the time of the depression the appearance of nucleic acid-components in the contents of the anthers can be shown by paper-chromatography. It is assumed, that a transfer of DNA takes place from the tapetum to the microsporocytes by means of the DNA-components.

LITERATUR

Anderson, E. and H. W. Beams. 1956. Journ. Biophys. Biochem. Cytol. *2*: 439–444.
Bryan, J. H. D. 1951. Chromosoma (Wien) *4*: 369–392.
Cooper, D. C. 1952. Am. Nat. *86*: 219–229.
De, D. N. 1956. Exp. Cell. Res. *12*: 181–184.
Dische, Z. 1930. Mikrochem. *8*: 4–32.
Eberhardt, F. 1952. Zs. Bot. *40*: 338.
Erickson, R. O. 1948. Am. Journ. Bot. *35*: 729–739.
Fiers, W. und J. Stockx. 1957. Naturwiss. *44*: 115.
Grun, P. 1956. Exp. Cell. Res. *10*: 29–39.
Hahn, L. und H. v. Euler. 1946. Ark. Chem. Min. Geol. A *22/23*: 1.
Hassenkamp, G. 1957. Naturwiss. *44*: 334–35.
Hassenkamp, G. und W. Liese. 1957. Exp. Cell. Res. *13*: 165–167.
Huskins, C. L. 1949. Proc. 8th Intern. Congr. Genetics. Hereditas (Lund): Suppl. 274–285.
Huskins, C. L. 1948. Journ. Hered. *39*: 310–325.
Huskins, C. L. 1950. Journ. Hered. *41*: 13–18.
Huskins, C. L. and N. Steinitz. 1948. Journ. Hered. *39*: 66–77.
Huskins, C. L. and N. Steinitz. 1948. Journ. Hered. *39*: 34–43.
Kandler, O. C. Zehender und J. Müller. 1956. Arch. Mikrobiol. *24*: 219–249.
Koller, P. C. 1951. Symp. Soc. exp. Biol. *1*: 270–290.
Linskens, H. F. 1955a. Papierchromatographie in der Botanik. Berlin-Göttingen-Heidelberg. 1–30.
Linskens, H. F. 1955b. Papierchromatographie in der Botanik. Berlin-Göttingen-Heidelberg. 142–153.
Linskens, H. F. 1956. Ber. dtsch. Bot. Ges. *61*: 353–360.
Moses, M. J. and J. H. Taylor. 1955. Exp. Cell. Res. *9*: 474–488.
Oehlkers, F. 1956. Das Leben der Gewächse. Teil I. Die Pflanze als Individuum, Berlin-Göttingen-Heidelberg.
Ogur, M., O. Erickson, G. U. Rosen, B. Sax and C. Holden. 1951. Exp. Cell. Res. *2*: 73–89.
Plaut, W. S. 1953. Hereditas (Lund) *39*: 438–444.
Sparrow, A. H., V. Pond, S. Kojan. 1955. Am. Journ. Bot. *42*: 384–394.
Swift, H. 1950. Proc. Nat. Acad. Sci. *36*: 643–654.
Taylor, H. 1953. Exp. Cell. Res. *4*: 164–173.
Taylor, H. and R. McMaster. 1954. Chromosoma (Wien) *6*: 489–521.
Taylor, J. H. 1957. Am. Nat. *41*: 209–220.
Wallenfels, K. und W. Christian. 1953. Angew. Chemie. *65*: 459–461.
Webb, J. M. and H. B. Levy. 1955. Journ. biol. Chem. *213*: 107–117.
Welzel, G. 1954. Zs. Vererbungsl. *86*: 35–53.

Acta Botanica Neerlandica **7** (1958) 69–76

THE INFLUENCE OF THE pH ON THE GROWTH AND THE GEOTROPISM OF DECAPITATED AVENA COLEOPTILES SUPPLIED EITHER WITH INDOLEACETIC ACID OR WITH INDOLEACETONITRILE

L. ANKER
(*Botanical Laboratory, Utrecht*)

(*received January 9th, 1958*)

Introduction

If the growth of a tissue when it is supplied from the outside with an acid auxin, proves to be either promoted or inhibited according to the pH of the medium, these effects might be due to a different degree of dissociation of the auxin, as the latter might influence the rate of its penetration into the cytoplasm.

The pH of the surrounding medium, however, may change in addition the acidity of the cytoplasm, and thus influence the activity of the enzymes which determine the rate of cell elongation.

As to the latter possibility, in earlier publications a complete independence of the internal pH of the external one has been defended by Albaum et al. (1937) and by Rietsema (1950), whereas a complete adjustment has been claimed by van Santen (1940). In more recent papers, however, the question of an adjustment of the internal pH is discussed with great caution (Simon and Beevers, 1952; Blackman and Robertson-Cuninghame, 1953). Probably one has to distinguish between a pH occurring in the greater part of the cytoplasm and the various H-ion concentrations prevailing in the different micro-environments that must be present inside the cytoplasm. The latter are possibly of primary importance for the auxin *action*, while the pH prevailing in the greater part of the cytoplasm might be essential for the auxin *transport*, e.g. in a process like the geotropic curvature, which is studied in the present paper. If the auxin transport in the lateral direction would be accomplished by the "geo-electric effect of Brauner", it would considerably be facilitated by the presence of auxin in ion-form.

A complete adjustment of the internal pH to that of the medium, on the other hand, would involve that, for instance at pH = 4, no more than 10 % of the indoleacetic acid inside the cytoplasm would be dissociated. This decrease in the amount of available anions could become a limiting factor in the geotropic reaction if this process really is caused by an unequal distribution of auxin by means of electric forces.

By using both a dissociable (IAA) and an undissociable auxin (indoleacetonitrile, abbrev. IAN) in the present investigation, it was tried to discriminate between the effect exercised by the pH of the medium on the dissociation of IAA and its possible effect on the pH of the cytoplasm.

A somewhat similar trend of thought was at the base of the experiments of Audus (1949) in which he studied the influence of the pH on root growth, and compared its inhibition by 2,4-D and by the non-acid inhibitor coumarin. With the latter substance root growth inhibition appeared to be independent of the pH.

An analogous result was obtained in our own experiments, for with the non-acid IAN no effect of the pH was found either on the growth of the coleoptiles or on their geotropism. With *limited* amounts of IAA, however, both processes were markedly influenced. With *abundant* IAA, on the contrary, no effect of the pH was visible. The results suggest that the influence of the pH must have been due entirely to its effect on the rate at which IAA penetrates into the cytoplasm.

Methods

A detailed description of the method has been given earlier (Anker, 1954, 1956). Decapitated Avena coleoptiles (1 mm removed) were kept horizontally or vertically for the experimental period of 75 minutes in aerated solutions of the required composition. Due to the aeration the solution was constantly stirred, which is advisable in experiments on the influence of the pH on a submerged tissue (Audus, 1949).

The solutions were made up from tap water, KH_2PO_4 (0.01 M), varying amounts of H_3PO_4 and either IAA or IAN. The pH, which did not change during the experiments, which lasted only 75 minutes, was measured potentiometrically.

The air humidity in the room in which the experiments were made varied from 90–96 %. The temperature was kept constant at 23° C. Only phototropically inactive orange light was admitted throughout the cultivation of the test material as well as during the experiments.

The curvatures or the elongation of the coleoptiles were measured on shadowgraphs taken at the close of the experiment. The standard deviation of the mean curvature was about 1°. When the curvatures were small, due to a supra-optimal auxin-concentration, the variation was larger and the standard deviation sometimes exceeded 2°. The measurements of the elongation always showed a great uniformity. The standard deviation of the mean increase often remained in the vicinity of 5 % and seldom reached 10 %. Each experiment was at least twice repeated. In each of the Figures 1–4 the results of one typical experiment are shown.

Results

Lowering the pH inhibits geotropic curvature in experiments with IAA

In previous experiments (Anker, 1954, 1956) it was found that a 0.075–0.1 mg/l concentration of IAA is about optimal for the regulation of the geotropic reaction of decapitated coleoptiles at

$pH = 7.8$. If, however, as was done in the present experiments, the acidity of the medium is increased to a $pH < 6.1$, then the geotropic curvature rapidly decreases at the above-mentioned IAA concentration. This is shown in Fig. 1.

At pH-values > 7, IAA is completely dissociated, whereas the percentage of non-dissociated IAA rapidly increases in media with a pH below 6 (see Fig. 1). It is often assumed that the auxin molecule penetrates easier into the cytoplasm than the anion does. Since the geotropic reaction is confined to a narrow range of auxin concentrations (ANKER, 1956), the observed inhibition might be due to a rise of the internal IAA-level above the optimum value for the geotropic curvature. This supposition was confirmed by the results of the following experiments.

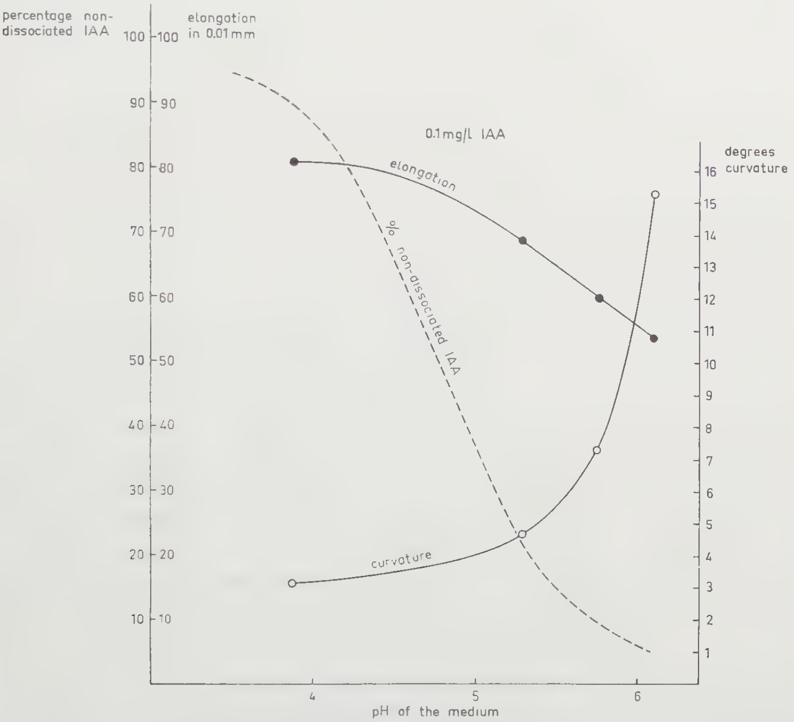


Fig. 1. The effect of pH on elongation and geotropism; 1 mm decapitation; 0.1 mg/l IAA.

Effect of the pH on the IAA-optimum for the geotropic reaction

In these experiments the relation between curvature and concentration was investigated at $pH = 4.1$ and at $pH = 7.8$. Although the shapes of the curves (Fig. 2) from which the relation is seen, show some difference, it is evident that at $pH = 4.1$, the concentration needed for maximal response was only one third of that required at $pH = 7.8$.

A shift of the optimal IAA concentration to a lower value, however, may theoretically be due (1) to an actual increase of the amount of IAA that penetrated into the cytoplasm (as suggested above), and (2) to an increase of the sensitivity of the cytoplasm to auxin, or to both these causes.

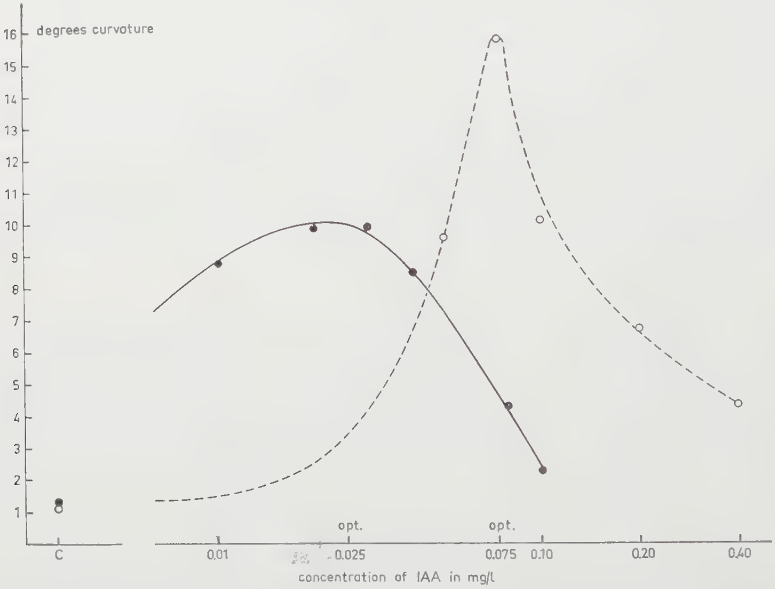


Fig. 2. The effect of pH on the IAA-optimum for the geotropism. ●—● pH = 4.1 ○—○ pH = 7.8.

No effect of the pH on the IAN-regulated geotropic reaction

To discriminate between these possibilities, experiments were included with indoleacetonitrile. As the auxin does not dissociate, the pH of the medium does not affect the growth substance outside the cell. Fig. 3 shows that when IAN was added in a range of concentrations, at pH = 4.1 as well as at pH = 7.8, the maximal geotropic curvature occurred at the same IAN concentration (0.0075 mg/l).

This result strongly suggests that neither the penetration of IAN into the cytoplasm nor the sensitivity of the cytoplasm to IAN is changed by lowering the pH of the medium from 7.8 to 4.1.

Effect of the pH on straight growth

As a further test of the hypothesis, that the effect of lowering the pH on the geotropism of the coleoptiles in the experiment with IAA is due to a facilitation of its penetration into the cytoplasm, the way in which the rate of *straight growth* depends on the acidity of the medium, was investigated. If our hypothesis is right this process should not be influenced (in any case not promoted) when IAA is supplied in abun-

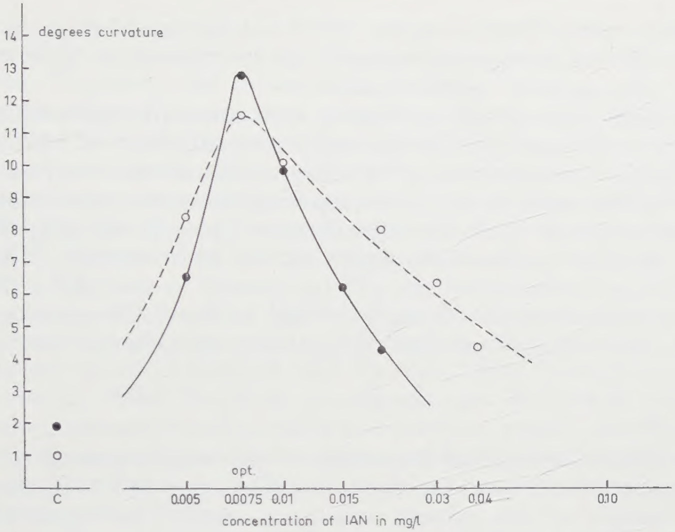


Fig. 3. The optimal IAN concentration for geotropism is not influenced by the acidity of the medium. ●—● pH = 4.1 ○—○ pH = 7.8.

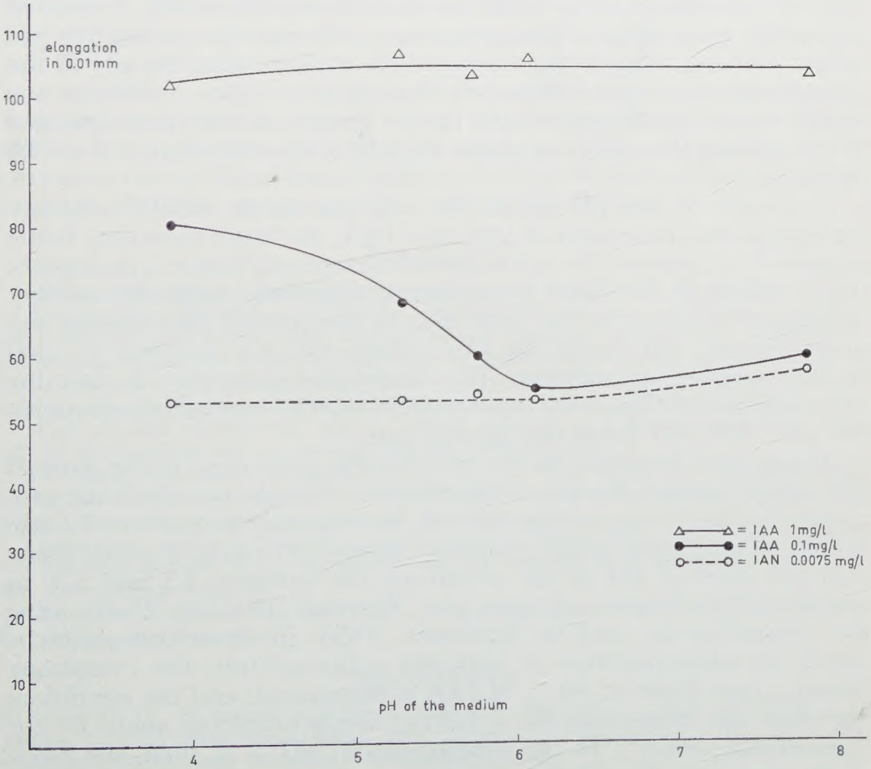


Fig. 4. Effect of the external pH on the straight growth at sub-optimal and supra-optimal IAA concentrations and at a sub-optimal IAN concentration.

dance, nor when IAN is added. With sub-optimal concentrations of IAA, on the contrary, a stimulation of the growth is to be expected because of improved permeation.

The results were in full agreement with these anticipations (Fig. 4).

At pH = 7.8 and 6.1 the 0.1 mg/l concentration of IAA and the 0.0075 mg/l concentration of IAN were of equal activity just as these concentrations were found to be equivalent in the regulation of the geotropic reaction in earlier experiments. At a lower pH, however, growth became considerably faster in the IAA solution. There was no significant influence of the pH on growth in the IAA solution of supra-optimal strength (1 mg/l). Also in the IAN solution whose strength was sub-optimal for the growth, no effect of the pH was observed.

DISCUSSION

The effect of the pH of the medium on straight growth as well as on the geotropic curvature of the coleoptiles appeared to be dependent on the nature of the growth substance used. The hypothesis that in the experiments with IAA the pH merely facilitates the penetration of this acid auxin by reducing the degree of dissociation, seems to explain the results satisfactorily. This view is corroborated by the fact that in the experiments with the non-dissociated IAN, it proved impossible to produce a change in these processes by varying the pH of the medium. This latter result would indicate that the pH of the cytoplasm is not much affected by that of the medium, otherwise one would expect a difference in the rate of growth and in the magnitude of the geotropic curvature when the pH is lowered from 7.8 to 3.8 or 4.1.

A change of the pH inside the cell, moreover, might influence the enzymatic conversion of IAN into IAA, the latter substance being supposed to represent the active growth promoting factor. The absence of pH effects in the IAN experiments, therefore, might be another argument in favour of the view that in the present short-lasting experiments the pH inside the protoplasm was not changed by that in the surrounding solution. The alternative possibility is that the conversion of IAN is a very rapid process, which, even at an unfavourable pH, does not limit the growth rate.

As has been developed in the introduction, a decrease of the internal pH might hamper the lateral distribution of auxin on which the geotropic reaction is supposed to depend, by reducing the amount of auxin anions. A protection of the cytoplasm against pH-changes would mean that the internal pH of the cytoplasm lies between 5.5 and 5.9, as was found by ALBAUM, KAISER and NESTLER (1937) in *Nitella* using the potentiometer, and by RIETSEMA (1950) in *Avena* coleoptiles by means of micro-injection of indicator solutions into the cytoplasm. Within these limits ca 90 % of IAA is dissociated, and the conditions therefore are favourable for a lateral displacement of auxin by the "geo-electric effect". In the experiments at pH = 4, then, the auxin molecules, once they had arrived inside the cytoplasm, would dissociate at this pH of ca. 5.7. "The undissociated molecules could have served merely as a form which readily penetrated the cytoplasm" (Erickson, Wedding and Brannaman (1955).

In intact coleoptiles auxin is produced in the tip. Its transport from cell to cell will be much facilitated by the presence of plasmodesms, since by the aid of these structures it need not leave the protoplasm, and a repeated penetration of the comparatively slower permeating auxin anions into successive protoplasts is evaded. The meaning of plasmodesms for the transport of salts has been stressed by Arisz (1956).

In recent papers mention has been made of a selective inhibition of geotropism by substances that inhibit growth to a much smaller degree or not at all (Mentzer and Netien, 1950; Mentzer, Molho and Pacheco, 1950; Te–May, Hamilton and Bandurski, 1956). A similar phenomenon was described in a previous paper (Anker, 1956), where it was caused by a slight increase of the external auxin concentration, and has also been found in the present experiments (see Fig. 1) after lowering the pH.

In contrast to the view of Te–May e.a., that a disproportional inhibition of geotropism and growth requires a modification of the Cholodny–Went-theory, the present author regards the selective inhibition observed in his own experiments as a confirmation of the above-mentioned theory. For, a proportionality of the rates of curving and of elongation would mean a maximal geotropic response at auxin concentrations which are optimal for growth. Since, however, the maximal curvature is carried out at concentrations which are *suboptimal* for the process of elongation (Anker, 1956), all results of the present series of experiments support the hypothesis that the geotropic reaction depends on an unequal auxin distribution.

SUMMARY

The *promoting* effect of an increased acidity of the medium on the straight growth of decapitated coleoptiles as well as the *inhibiting* effect on their geotropic curvature, that are observed when these coleoptiles are supplied with *limiting* amounts of indoleacetic acid, are to be ascribed to a decrease in the degree of dissociation of this auxin and to the circumstance that it penetrates more easily into the protoplasm in the molecular form, with the result that the internal auxin level is raised.

With *abundant* indoleacetic acid — which prevents a geotropic reaction — the pH of the medium did not change the rate of elongation.

The above conclusion is supported by the fact that when the non-dissociated indoleacetonitrile was supplied to the coleoptiles a decrease of the external pH from 7.8 to 3.8 produced no effect on these processes.

From these results it is further concluded that during the experimental period (75 minutes) the pH of the cytoplasm was not affected by a change of the pH of the surrounding solution.

The author is indebted to Prof. Dr. V. J. Koningsberger and to Dr. H. P. Bottelier for stimulating discussions.

REFERENCES

Albaum, H. G., S. Kaiser and H. A. Nestler. 1937. Am. J. Bot. *24*:513.
Anker, L. 1954. Proc. Kon. Akad. Wet. A'dam *C 57*:304.
Anker, L. 1956. Acta Botanica Neerlandica *5*:335.
Arisz, W. H. 1956. Protoplasma *46*:5.

AUDUS, L. J. 1949. New Phytologist *48*: 97.

BLACKMAN, G. E. and R. C. ROBERTSON–CUNINGHAME. 1953. New Phytologist *52*: 71.

ERICKSON, L. C., R. T. WEDDING and B. L. BRANNAMAN. 1955. Plant Physiology *30*: 69.

MENTZER, C., D. MOLHO et H. PACHECO. 1950. Bull. Soc. Chim. Biol. *32*: 572.

MENTZER, C. et G. NETIEN. 1950. Bull. Mens. Soc. Linn. Lyon *19*: 102.

RIETSEMA, J. 1950. Doct. Thesis, Utrecht.

SANTEN, A. M. A. VAN. 1940. Doct. Thesis, Utrecht.

SIMON, E. W. and H. BEEVERS. 1952. New Phytologist *51*: 163.

TE–MAY, T. C., R. H. HAMILTON and R. S. BANDURSKI. 1956. Physiologia Plantarum *9*: 546.